Literature review

Vitamins, minerals and trace elements

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Preface

The use of complementary feeding stuffs in farm animals can in some situations contribute to good health and production. The appropriate use of complementary feeding stuffs or veterinary medicines with vitamins, minerals and trace elements can be supported by the use of literature.

This literature review describes the literature that is known to Dopharma and concerns the active ingredients included in the complementary feeding stuffs marketed by Dopharma. These include vitamins, minerals and trace elements. This is a general discussion which does not take into account the levels of certain components that are needed to achieve an effect in comparison to the concentration in the complementary feeding stuffs.

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7.1.1 Biotin

In broilers a concentration of 150 µg /kg feed is recommended, but several authors have advised higher concentrations of at least 170 µg/kg, but sometimes even 250-300 in specific situations [1]. For layers the requirement is 0.1 mg/kg. The recommendation for breeders is 0.10-0.16 and 0.10-0.20 mg/kg feed for chicken and turkeys respectively [2].

Biotin is a coenzyme essential for gluconeogenesis, lipogenesis and the elongation of essential fatty acids. It is important for the reproductive and nervous systems and for the thyroid and adrenal glands [3]. Deficient flocks show a poor growth, poor feathering and elevated mortality. Clinical signs are dermatitis of the feed and skin. In breeders hatchability is decreased and malformations can be found in the progeny[4].

The requirement of biotin in chicken breeders has been studied by several authors. Whitehead *et al*. showed that a sufficient amount of biotin of 16 µg/day for breeders is needed to guarantee growth potential and vitality of the chicks[5]. Brewer & Edwards advice a to use at least 150-252 µg/kg feed for good hatchability and vitality of the chicks[6]. The hatchability increased from 84% to 89% when the level of biotin in the feed in increased from 165 to 440 µg/kg [3]. The supplementation of biotin at a concentration of 0.45 mg per liter of drinking water resulted in an increase in production from 73.5% to 87.8%. Hens from a low-fertility line also showed an increase (8.4%) in fertility rate, but this effect was not seen in hens from a high fertility line[7].
In Barroeta *et al.* not only laying and hatchability are mentioned as being dependent on biotin, but also mortality during the last week of incubation and the occurrence of bone deformities in the progeny[3].

In turkey breeders supplementing the feed with more (0.75 mg/kg vs. 0.178 mg/kg feed) biotin results in increase in egg production and an improvement in hatchability, especially at the end of the production period (week 50-54). In the same article it is however mentioned that results could not be repeated [8]. Robel did find similar results; when the concentration in feed was increased to 720 µg/kg during week 50-54 the hatchable egg production increased by 22% and the chick hatchability by 10% [9]. During the initial and intermediary phases of reproduction only a concentration of 520 µg/kg was needed to achieve maximum hatchability.

In broilers a deficiency of biotin is related to hypoglycaemia, dermatitis, growth reduction, foot pad deformities and alterations in bone formation[1]. A diet with 400 µg biotin per kg feed was sufficient to prevent problems such as varus deformations, food pad laesions, a shorter tibiotarsus and a lower bone density[10]. The occurrence of tenosynovitis and twisted feet due to a reovirus infection is significantly reduced in chicken fed biotin at a dosage 200% as high as recommended by the NRC [11].

Whitehead *et al.* clearly proved that fatty liver and kidney syndrome occurs as a result of biotin deficiency. This syndrome occurred in particular when broiler diets were low in fat and protein[12].

In Cepero & Pérez several studies are described in which the daily weight gain and the feed conversion rate are improvement when the diet of broilers is supplemented with additional biotin. The amount of biotin used in these studies ranged from 200 µg/kg to 550 µg/kg feed[1].

The occurrence of acute death syndrome (ADS) in broilers could not be influenced with biotin in concentrations of 20 or 100 µg per day [13].

Marginal levels of biotin may lead to the appearance of fatty liver or kidney syndrome in layers, especially when there is a low level of fat in the diet and lipogenesis is necessary. This situation may be aggravated by stress. It seems that biotin may prevent fatty liver syndrome but once sings have appeared, supplementing biotin does not seem to have beneficial effects on the accumulation of lipids in the liver. Depending on the biotin content of the diet, the supplement should contain up to 15 mg biotin/kg [14].

In layers supplementing biotin (110 µg/kg) did not influence egg production, egg weight, feed intake, bodyweight, the weight of the liver or the amount of fat in the liver [15].

8.1.1 Choline

The recommendation for choline in layers is 470-1300 ppm. For broilers and turkeys the recommended concentrations in feed are respectively 750-1300 IU/kg feed and 800-1600 IU/kg feed [2]. Recommendations for breeders found in literature vary greatly from 250 to 1000 mg/kg [3]. Birds can synthesize choline in the liver from the amino-acid methionine. The ability is however insufficient to meet the requirements in conditions of intensive production, even with adequate levels of methionine [14]. The requirement of choline decreases with age as the synthesizing capacity increases; the requirement is highest in the starter phase. Slower growing broilers also have a lower requirement than conventional broilers [1].

As a structural component of lecithin, choline plays an essential role in the formation of very low density lipoproteins assigned to incorporating and mobilizing the triglycerides present in the liver. Lecithin deficiency is associated with an accumulation of fat in the liver and a decrease in the quantity of egg yolk. Supplementing choline does however not increase egg production or hatchability or improve fertility [16]. In Barroeta *et al.* several studies are described in which choline supplementation results in a decreased concentration of fat in the liver. It is also suggested that choline may be particularly interesting in conditions of heat stress since the deposition of fat in the liver increases significantly at higher temperatures [14].

Supplementing higher concentrations of choline does affect the size of the egg in breeders, but not the total choline deposition in the egg or the embryonic development [3]. An increased egg size was also found by Couch & Grossie [17] and Tsiagbe *et al.* [18]. These authors also found an improvement of the feed conversion rate, but no effect on egg production.

In broilers several experiments have verified that the addition of choline produces significant improvements in growth, feed consumption and feed conversion index, and that the dose-response relationship was linear, although in some cases this was only found in the starter phase. The concentrations at which this is achieved are however higher than the NRC recommendations and dependent on the composition of the feed [1].

In turkeys choline supplementation has shown to be effective to increase growth between 8 and 12 weeks of age, especially if methionine levels are low. This effect was however not achieved in the period between 4 and 8 weeks of life [1].

The choline requirement of ducks is probably 810-823 mg/kg feed when only weight gain and feed intake are evaluated. A concentration of 1182 mg/kg choline in the feed however resulted in lower total lipid and triglyceride concentrations in the liver and a higher phospholipid concentration. This concentration also reduced the incidence of perosis to zero [19, 20].

8.1.2 Folic acid

The NRC recommendation for folic acid in broilers is 0.55 mg/kg feed, but this recommendation has not been changed for over 25 years and later authors have suggested levels up to 3 mg/kg [1, 2]. For layers it is recommended to use 0.25mg/kg feed. In broiler breeders the NRC recommends the incorporation of folic acid at a concentration of 0.35 mg/kg feed. For turkey breeders a higher concentration is recommended: 0.7-1.0 mg/kg feed[2].

There is one study in which the folic acid requirement of broilers was determined at 0.12-1.69 mg/kg feed [21].

Folic acid is essential in the transfer of monocarbonate units in metabolic processes, affecting the synthesis of purines and pyrimidines, which make up the nucleic acids needed for cell division. It is also involved in the metabolism of serine, glycine, histidine, methionine, choline and thiamine [3].

In laying hens folic acid is needed for the normal development of the oviduct. Hens which have been deficient of this vitamin will have a defective deposition of albumen [3]. Husseiny *et al.* demonstrated that the egg weight can be increased when folic acid is supplemented at a concentration of 12 mg/kg diet[22].

The use of folic acid in turkey breeders results in an increased concentration of this vitamin in the egg, which possibly improves the weight of the poults at hatching as well as the subsequent growth [3].

Folic acid supplemented at concentrations twice as high as the NRC recommendation results in a decrease of pathological laesions caused by reovirus infections [11]. Low levels of folic acid in diets high in protein increases the incidence of foot problems [1].

8.1.3 Niacinamide

The NRC guideline does not mention a requirement for niacinamide, but there are requirements for niacin. Niacinamide is an amide of niacin and both possess the same biological activity. For broilers the NRC recommends incorporating 35 mg/kg feed. In turkeys the requirement is higher: 60 mg/kg[2]. Ruiz & Harms found a requirement for broilers (3-7 weeks of age) of 22 mg/kg feed [23]. For the age of 1-21 days they found a requirement of 32 mg/kg feed [24]. The requirement in layers is 10 mg/kg[2]. The requirement in pullets (age 1-6 weeks) is 25.1 mg/kg feed [25]. The requirement for broiler breeders is 10 mg/kg feed, for turkey breeders this is higher: 40 mg/kg feed[2]. Whitehead however suggests basal rations should contain 80-100 mg niacin per kg for chicken and 70 mg/kg for turkeys up to 12 weeks of age [26].

Niacin and niacinamide are functional parts of the coenzymes niacinamide adenine dinucleotide (NAD) and niacinamide adenine dinucleotide phosphate (NADP), involved in cellular respiratory processes [3]. Furthermore, it assists in the release of energy from feedstuffs and in its delivery to the body's cells [14].

In a study with laying hens it is shown that supplementing niacin at a concentration of 44 mg/kg feed results in an increase in egg production. The increases however become significant when 66 mg/kg or more was supplemented. Shell quality also increased in supplemented hens [27]. In layers niacin is also used to influence liver metabolism; liver weight, fat content of the liver [15].

An increased niacin gift in pullets above the requirement (25.1 mg/kg feed) does not influence body weight, sexual maturation or the egg production later in life [25]. In another study niacin did also not influence production parameters in pullets [28].

Some authors indicate that supplementing niacin at a concentration of 66-132 mg/kg improves the quality of the egg shell and lowers mortality both in the embryo and the breeder itself [3]. In breeders 23.6 mg niacin per kg feed is enough for maximizing production, fertility and hatchability. Body weight of the breeders and egg weight did however increase when the concentration of niacin in the feed was increased with 8.4 or 16.7 mg/kg [29].

The effect of niacin on growth rate in broilers in contradictory; one study describes an increase in bodyweight when a basal diet with 33 mg niacin/kg was supplemented with 33-66 mg/kg, but in another study no significant effects were found when supplementing niacin [1].

8.1.4 Vitamin A / Retinol

In broilers and turkeys it is recommended to incorporate vitamin A in levels of respectively 1.500 and 5.000 IU/kg feed. The recommendation for breeder and layer chickens is 3.000 IU/kg feed[2], but the more recent recommendations are higher with 8.000-12.000 IU/kg feed [3].

It is unlikely that a vitamin A deficiency occurs because animals can synthesize vitamin A out of β-carotene. In situation of stress and illness or due to mycotoxins, the efficacy of this conversion may however be impaired [1].

Vitamin A has fundamental importance in several metabolic processes and has important effects on sight, bone growth, the quantity and quality of semen produced and the growth and differentiation of epithelial tissues of the reproductive system and the embryo. It is thus very important for the development of young animals and fertility. Among the first symptoms of a deficiency of vitamin A are a decrease in sexual activity in males and failure of spermatogenesis, accompanied by a reduction in fertility and in the number of hatched eggs. When breeders consume marginal amounts of vitamin A, their progeny will have lower reserves. Adequate vitamin A in the hatching egg results in better growth rate, greater capacity for immune response and prevents pigmentation problems in the chicken [3]. A deficiency of vitamin A in layers is characterized by a reduction in egg production, blindness and keratinization of the epithelium [14]. The use of minimum levels in broiler feed might result in marginal deficiencies characterized by changes in skeletal development, a fall in the numbers of antibodies, a reduction in cellular immunity, rapid depletion of hepatic reserves and a drop in muscle glycogen reserves. More pronounced deficiencies can result in severe disruptions in respiratory and intestinal epithelia and in extreme cases in blindness and death [1]. Other symptoms which can be found in deficient chicks are ataxia, xeropthalmia and a chronic purulent conjunctivitis. In layers blood spots can be found on the eggs. Since it is important for the mucosa of the gastrointestinal tract, flocks with a vitamin A deficiency show a high prevalence of *E.coli*, other bacterial infections, endoparasites and coccidiosis [4].

Vitamin A (8000 IU/kg feed) is important for the local immunity in the intestines of broilers when they are challenged with *Eimeria acervulina* [30]. Also Peek *et al* [31] describe a positive effect of vitamin A on growth performance, mortality and oocyst excretion in chickens infected with *E. acervulina* or *E.tenella*. In this article a vitamin A deficiency is related to an increased susceptibility to E. acervulina due to a reduction in gut immunity and the systemic immune response.
Lessard *et al*. showed that chicks with a vitamin A deficiency primarily showed a Th1 immune response, while chicks which were given sufficiently high levels of vitamin A in their feed (15.000 IU/kg feed) primarily had a Th2 immune response[32]. In a study done by Lin *et al*. results indicated that vitamin A supplementation in commercial layer diets under heat stress was beneficial to laying performance and immune function in the period after vaccination. The effects on antibody titres were found with levels of 6.000 and 9.000 IU/kg feed [33]. The recommended amount of vitamin A for optimum functioning of the immune system are not the same for every disease: in case of NCD an

increase in the proliferation of lymphocytes, macrophages and specific antibodies in chicken and turkeys was seen with 18.999 IU; when chicken were infected with *E.coli* the optimum concentration was 60.000 IU/kg; when vaccinating against chicken pox or NCD a higher concentration of specific antibodies was produced when the concentration was 6.700 IU/kg [1].

Supplementing vitamin A at a concentration of 8000 IU/kg feed to laying hens decreased the negative effects of heat stress on the production of eggs. Vitamin A can possible diminish the oxidative reactions and negative influence on the immune system caused by heat stress [34].

In broilers supplementing vitamin A (15.000 IU/kg feed) resulted in an increase in weight gain, feed efficacy, carcass quality and MDA (indication for oxidative stress) concentration in serum [34].

8.1.5 Vitamin B_1 / thiamine

The NRC requirement for broilers and turkeys are respectively 1.8 and 2.0 mg/kg feed. For layers the recommended concentration in feed is 0.7 mg/kg. The NRC recommends incorporating vitamin B_1 at a concentration of 0.7 for chicken breeders and 2.0 for turkey breeders[2].

Vitamin B_1 is important in the carbohydrate metabolism and especially important for the energy metabolism in the heart and nervous system. When feed is given with high carbohydrate content, additional vitamin B_1 should be supplemented. Vitamin B_1 is also necessary for the synthesis of nucleic acid and of acetylcholine and is therefore essential in the transmission of nervous impulses. The availability of vitamin B_1 may be reduced by the occurrence of mycotoxins, thiaminases or therapeutic substances (e.g. amprolium) in the feed [1, 4, 14].

Inadequate supplies of vitamin B_1 in breeder hens cause high mortality in embryos and results in chicks with polyneuritis [3]. The most important signs occur in ten to twenty day old chicks and comprise abnormal retraction of the head, also known as star gazing and incoordination [4].

Even though chicks can synthesize vitamin B_1 from 5-7 days of incubation, the quantity deposited in the egg is important for the first few days of life. The majority of recommendation tables recommended dosages of 1.5-2.0 mg/kg feed, which is at least twice as high as the recommendation from the NRC [3].

In a very old study vitamin B_1 concentrations between 0 and 1.67 ppm have been investigated. With long term use of 0.55 ppm, symptoms of polyneuritis appeared which could be alleviated with parenteral injections of thiamine. The best productive yields were achieved with 1.25 ppm of vitamin B_1 [14].

In broilers using concentrations higher than recommended by the NRC has resulted in an improvement of weight gain and conversion rate. In young turkeys an increased concentration of vitamin B_1 resulted in a reduction of mortality [1].

In one study the vitamin B_1 requirement of broilers was determined by studying concentrations of 2-32 mg/kg. When a concentration of 8 mg/kg was used, the plasma concentration started to increase, but the plasma concentration increased further until concentrations of 32 mg/kg were used. The requirement is supposed to be more or less 8 mg/kg feed, but more research has to be done to confirm this [35].

8.1.6 Vitamin B_2 / riboflavin

The NRC recommends use of vitamin B_2 in a concentration of 3.6 and 3.6-4 mg/kg feed for respectively broilers and turkeys. In laying hens the concentration advised by the NRC is 2.5 mg/kg feed and for broiler and turkey breeders recommendations are respectively 3.6 and 2.5-4.0 mg/kg feed[2].

Vitamin B_2, in the forms of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), is important for over 100 enzymes involved in the metabolic oxidation processes of fat and protein. Recommendations of this vitamin increase when the feed contains high concentrations of these nutrients [3]. Besides, vitamin B_2 also plays an important role in maintaining the integrity of mucous membranes and the nervous system [1].

A deficiency of riboflavin is also called curled toe paralysis. This symptom occurs particularly at the age of 10-30 days and comprises a rotation of the legs. Also a decreased growth rate and poor feathering can be found. Breeders deficient of this vitamin show a decreased egg production and hatchability [4].

The growth of broiler chicken at the age of 1 to 21 days was stimulated when the concentration of vitamin B_2 in the feed was increased with 2 mg/kg. There was however no influence on the feed intake. These authors recommend a total concentration of 5 mg/kg feed [35]. Also Deyhim *et al*. found that doubling the concentration of vitamin B_2 compared to NRC recommendations results in an increase in live weight of 6% and 5% when broilers were subjected to heat stress. The feed conversion rate and mortality fell by 2% and 6%.

A deficiency of vitamin B_2 in broilers can lead to paralysis, a high mortality and a decreased growth rate. This was seen for concentrations of 2.6 mg/kg feed. The minimum recommendation according to these authors is 3.6 mg/kg feed, but to prevent paralysis from occurring it is better to use 4.6 mg/kg feed [36]. Other signs of a vitamin B_2 deficiency in broilers are poor plumage, a decrease in growth rate and feed conversion, paralysis, claw curvature, lowering of resistance to heat stress, enteritis and diarrhea and an increase in mortality in the first week [1].

In layers it has been shown that an increase in vitamin B_2 concentration in feed from 2.5 to 8.5 mg/kg resulted in a significant increase in egg production [37]. Kirichenko also showed an increase in egg production when the concentration of vitamin B_2 increased with 25% above the recommendations [14]. There was however also another study by Flores & Scholtyssek [14] in which increasing vitamin B_2 from 1.7 to 9.7 mg/kg did not result in an increase in egg production.

A deficiency of riboflavin reduces the ability of hens to deal with heat stress. It would be advisable to give the hens more vitamin B_2 during heat stress, but also in cases of immunological stress, because of its involvement in antibody synthesis [38].

The use of vitamin B_2 in breeders has an obvious influence on the vigour and survival capacity of the chicks borne. Concentrations which are at least twice as high as the concentrations recommended by the NRC (7-12 mg/kg feed) are therefore recommended in practice [3].

8.1.7 Vitamin B_5 / D-panthenol

The NRC recommends use of vitamin B_5 in a concentration of 10 mg/kg feed for both broilers and turkeys. In laying hens the concentration advised is 2 mg/kg feed[2], but an old study already mentioned higher levels (4.8 ppm) for good sexual maturity and future performance [39]. For broiler and turkey breeders recommendations are respectively 7 and 9-16 mg/kg feed[2].

Vitamin B_5 is part of coenzymes which are important for the utilization of nutrients, synthesis of fatty acids and participation in the citric acid cycle, as well as in the energy-yielding oxidation of fats, carbohydrates and amino-acids [3]. The main damages caused by a vitamin B_5 deficiency involve the nervous system, adrenal insufficiency and the skin. Symptoms are unspecific: dermatosis, changes in feathers and a reduction in reproductive yield [14].

There is one study known in which the effect of vitamin B_5 on broiler chickens has been investigated. Supplementing the chicks wit 0 – 14.4 mg/kg feed did not influence growth or the feed conversion rate [40]. Other studies in both broilers and turkeys have revealed the same result [1].

In breeders the concentration of this vitamin is important for the hatchability and the vitality of the chicks. A concentration of 4 mg/kg is needed to prevent losses in reproduction, but higher dosages of 8 or 20 mg/kg are needed to ensure a good hatchability and good vitality of the chicks [41].

8.1.8 Vitamin B_6 / pyridoxine

For broilers and turkeys a concentration of respectively 3.5 and 4.5 mg/kg feed is minimally advised. The recommendation for layers is 2.5 mg/kg. In breeders the recommends a concentration of 4.5 mg/kg feed. In turkey breeders the recommendation is 3-6 mg/kg feed[2].

Vitamin B_6 is one of the numerous enzymes involved in the metabolism of nutrients, especially as a cofactor of enzymes important for catabolism and interconversion of amino acids. Inadequate vitamin B_6 will lead to inefficiencies in the utilization of protein in the diet, accompanied by a reduction in the capacity of nitrogen retention and an increase in nitrogen excretion [3]. A deficiency will also result in an abnormal gait, convulsions and a bilateral enlargement of the hock joints, sometimes accompanied by displacement of the gastrocnemius tendon [4].

The requirement increases with higher dietary protein content and for feeds formulated to contain high levels of energy from fat [14].

In breeders inadequate consumption of vitamin B_6 causes a reduction in the production of eggs and their hatchability. In studies with turkeys an increase in hatchability could not be obtained with supplementing vitamin B_6, but this might have been due to the fact that the basal feed already contained a high concentration of vitamin B_6 [3].

Layers that are given feed deficient in vitamin B_6 in the short term experience a loss in body weight, a reduced feed intake and a marked reduction in body fat reserves. In the long term the reproductive organs alter and the egg production ceases. Animals which are deficient of this vitamin are also less capable of handling stress caused by heat or cold [42].

Broilers given additional vitamin B_6 were better capable of handling thermal stress than control animals. This effect is improved when vitamin B_6 is combined with L-carnitine. This synergy may be caused by the potential of both substances to regulate energy metabolism and modulate oxidative stress [43].

Vitamin B_6 is also linked to the prevention of feed problems because of its importance for the integrity of the conjunctive tissue matrix and the development of the skeleton [1].

8.1.9 Vitamin B_{12} / cyanocobalamin

The requirement mentioned is 0.01 mg/kg for broilers, 0.003 mg/kg for turkeys, 0.004 mg/kg for layers, 0.008 mg/kg for broiler breeders and 0.003 for turkey breeders[2].

Vitamin B_{12} is involved in the metabolism of fatty acids and the synthesis of proteins [1]. It is also important for the normal development of erythrocytes and a deficiency causes macrocytic anaemia. It is the only vitamin that is synthesized only by microorganisms, meaning that products of vegetable origin are almost devoid of this vitamin [14].

It is generally believed that a deficiency of this vitamin will not affect commercial egg production, but a decrease in egg weight has been reported in 1950. Also in a long term study it was shown that

supplementing 8 µg/kg resulted in better production parameters such as egg production, egg weight, shell thickness, hen weight and optimum hatchability [14].

Patel & McGinnis studied the effects of vitamin B_{12} supplementation (0.01 mg/kg) in breeders. There was no effect on fertility, but hatchability, chicken viability and growth of the chicks did improve[44]. Supplementing the breeders with vitamin B_{12} was in this case more effective than supplementing the chickens after hatching. Panic *et al*. showed that vitamin B_{12} supplementation (20µg/kg) results in an increase in hatchability of 20% [3].

8.1.10 Vitamin C / ascorbic acid

The NRC does not advice minimal recommendations for vitamin C in poultry because birds can synthesize their own vitamin C. It is however mentioned that the supplementation of vitamin C during stress full periods can have positive effects[2].

Vitamin C is involved in fundamental biological and metabolism processes such as the conversion of vitamin D_3 to its active form, biosynthesis of collagen, absorption of minerals (iron), control of glucocorticoid synthesis, simulation of phagocytic activity and as an antioxidant on a cellular level [1].

Vitamin C has a positive influence on the intestinal morphology (thickness of lamina propria and length and width of villi) in broilers with hypertension (height induced). Amounts supplemented were 400-1200 mg/liter drinking water [45].

Vitamin C in a concentration of 150-450 mg/kg resulted in a decreased incidence of ascites in broilers given feed with high concentrations of sodium chloride [46].

Even though vitamin C stimulates the renal synthesis of the active form of vitamin D_3, it does not have any influence on the occurrence of tibial dyschondroplasia in broilers [47]. Supplementing layer pullets (250-300 ppm) also did not influence the formation and structure of bones at 15 weeks of age [48]or change the volume of medullary or trabecular bone at the end of the laying period [49].

During periods of heat stress the supplementation of vitamin C (250 mg/kg feed) resulted in an increase in weight gain [50] and 150 mg/kg feed influences the energy metabolism during heat stress [51]. Pardue *et al*. found that vitamin C (250-1000 ppm) supplementation results in a decrease in heat stress associated mortality, but without any effect on chick vitality [52]. The effect of vitamin C during periods of heat stress is also examined by Abidin & Khatoon. Vitamin C supplementation during this period had positive influences on immunity, feed intake, and body weight, the occurrence of oxidative stress, fertility, sperm quality and mortality [53]. In their book Barroeta *et al*. conclude that vitamin C (250-400 ppm) supplementation during periods of heat stress results in an improvement of survival, feed consumption, production and egg quality [14]. There are however also situations in which no influence of vitamin C on heat stressed chickens has been shown. The dosages recommended range from 200-250 to even 1000 mg/kg feed [1]. Curça *et al*. even mentioned concentrations of 2000 mg/kg feed to improve the weight of broilers kept at high temperatures by 12% at 4 weeks of age [54].

The use of vitamin C has also been recommended by several authors before and during a treat from pathogens. Stimulation of the immune system has been observed in several diseases: coccidiosis, infectious bronchitis, colibacillosis, Marek's disease, aflatoxicosis, other intoxications and ascites. The use of vitamin C will not prevent or cure disease, but most commonly results in a significant reduction in laesions and mortality, an improvement in cellular immunity, an increased amount of antibodies and an antioxidant effect. The concentrations used are most often 300-330 mg/kg. In severe cases concentrations of up to 1000 mg/kg have been proposed, but it has also been reported that concentrations above 400 ppm are less effective [1].

Also when applied before and after vaccination, vitamin C (5000 ppm) stimulated the production of antibodies as a response to the vaccination [14].

The use of 1000 ppm vitamin C in drinking water of day-old chicks after prolonged transportation significantly reduces mortality [1].

McKee & Harrison investigated that effect of vitamin C supplementation during periods with multiple stressors such as heat stress, beak trimming and coccidiosis. Supplementing 150 ppm in the feed improved production parameters significantly [55].

The authors who have evaluated the effects of vitamin C on production parameters of laying hens have found conflicting results. There are authors who have showed no effect with concentrations of 0-400 ppm and even 1000 ppm in feed or water. There are however also authors who do show an increase in egg production, especially during periods of heat stress, and an increase in egg weight and egg density [14].

In broiler breeders supplementing vitamin C (75 mg/kg feed) did not improve egg production, egg shell quality, fertility or hatchability [56]. Other authors did however find an improvement in egg production, in strength and thickness of the shell as well as better chick survival [57, 58]. The fertility of male chicken is improved when vitamin C is supplemented. Changes that have been observed are an increase in testicular weight (100 ppm vitamin C), an increase in volume of semen, number of spermatozoa per ejaculation and sperm motility (500 ppm vitamin C) [3].

In a study in ducks it was shown that adding 400 mg of vitamin C per kg feed resulted in an increase in growth and an improvement in the antioxidant status and humoral immunity [59].

8.1.11 Vitamin D_3 / cholecalciferol

The requirement mentioned is 200 IU/kg for broilers, 1100 IU/kg for turkeys, 300 IU/kg for layers, 300 IU/kg for broiler breeders and 1100 IU/kg for turkey breeders[2].

It is difficult to establish a good vitamin D_3 requirement for chickens, because the amount needed is influenced by the concentration of minerals such as calcium and phosphorus that are available to the animal. Besides, high levels of vitamin A and E may reduce the vitamin D_3 status. Also genetic differences between breeds influence the requirement [1]. It has been shown that vitamin D_3 supplementation is beneficial under both optimal and lower Ca and P inclusion levels in broiler diets, but achieves the best effect with low calcium levels [60].

Cholecalciferol is stored in the liver and adipose tissues in an inactive form. When vitamin D is needed, in the liver cholecalciferol is activated by hydroxylation into 25-hydroxycholecalciferol. This metabolite is carried to the kidneys and converted to 1.25-hydroxycholecalciferol (calcitriol), which is de active form in animals [14].

The active metabolite of vitamin D_3 (calcitriol) regulates the absorption, transport, deposition and mobilization of calcium together with parathyroid hormone. Increasing concentrations of vitamin D_3 result in an increased concentration of ionized and total calcium, a decreased concentration of sodium and phosphorus and an improved absorption and retention of phosphorus. A vitamin D_3 deficiency results in hypocalcaemia, hypophosphatemia, rickets (with an enlargement in the proliferative zone of the growth plate) and tibial dyschondroplasia [1]. Affected flocks usually show reluctance to move at the age of four to seven weeks together with swelling of the joints, a poor growth rate and poor feathering. In egg producing flocks osteomalacia will occur and the egg production will decrease. Also the quality of the egg shell decreases gradually [4]. There is some conflict in the concentration of

vitamin D_3 that should be used to gain optimum ash content of bones; Whitehead concluded that 1000-1250 IU/kg was not enough to totally prevent rickets or acquire maximum bone ash content[61]. In 2004 the same author concluded that the requirements of broilers up to 14 days of age to gain a good cortical bone quality range from 35 to 50 µg/kg feed with optimal dietary calcium and phosphorus levels. The requirements increase to 250 µg/kg feed to prevent TD [62]. Kasim & Edwards however did obtain maximum bone ash content with 1100 IU/kg [63]. When chickens are not exposed to ultraviolet light, the concentration of vitamin D_3 has to be increased to 1600 IU/kg [64]. In a recent study it was concluded that the supplementation of vitamin D resulted in a lower incidence of lameness in general and specifically bacterial chondronecrosis with osteomyelitis. This effect was found even though the vitamin D concentration in the basal feed was already 5500 IU/kg [65].

The prevention of bone problems in broilers is best done with supplementation of the breeder hens. When hens were supplemented with 2000 IU/kg (compared to 250 IU/kg), the incidence of tibial dyschondroplasia in the progeny decreased during the middle and final stages of production [66]. A combination of supplementing both breeders and broilers is also possible: Atencio *et al*. showed that broilers which came from hens given 2000-4000 IU/kg and were given 200-3200 IU/kg in their own feed showed best growth and highest bone ash content [67]. In another study from the same author concentrations of 2000-4000 IU/kg feed were determined as minimal requirement to minimize the occurrence of tibial dyschondroplasia and calcium rickets as much as possible [68].

When looked at growth, bone mineralization and excretion of minerals, the minimum vitamin D_3 requirement in broilers is 25 µg/kg [69]. Feed with high concentrations of vitamin D_3 (3500 IU/kg) decrease the occurrence of tibial dyschondroplasia in broilers.

Besides a decrease in bone abnormalities in the progeny, vitamin D_3 supplementation of the breeders also results in an increased growth rate of the progeny [66, 68].

Vitamin D_3 concentrations of 1500-3500 IU/kg positively influence the immune system of broilers. The mortality is however not different in supplemented and not supplemented broilers [70]. The role of vitamin D_3 is associated with cell medicated immunity since this vitamin is needed for the maturation and functioning of macrophages, especially during the first two weeks of life [1]. Vitamin D_3 has a dose –dependent immunomodulatory effect, with improvement of the Th2 cell response [60].

In laying hens vitamin D_3 supplementation (11.200-12.000 IE/kg feed) did not change egg shell quality or the concentration of vitamin D_3 in the eggs produced [71]. Other authors however did find that increasing the vitamin D_3 concentration from 250 to 4000 IU/kg resulted in a decreased percentage of broken eggs and an improvement in egg specific gravity [72, 73]. Studies by Frost *et al.* [74] and Faria *et al*. [75] showed that increasing vitamin D levels to respectively 500-1500 and 2500-3500 IU/kg resulted in an increase in egg production, consumption of feed, egg specific gravity, the percentage and weight of the shell and shell strength.

The requirement of broiler breeders when determined on the basis of egg production and progeny production parameters was 1400 IU/kg feed. In this experiment the chickens were housed in an environment deficient of UV light [76].The concentration of vitamin D_3 in the egg of breeders is important for the development of the progeny. When this concentration is too low, the transport of calcium from the eggshell via the chorioallantoic membrane to the bones is disrupted resulting in poorly calcified bones. When the problem is extensive, many chicks die at the end of the incubation period and the chicks that do hatch tend to be weak and have ossification problems. The supplementation of vitamin D_3 in breeders is most important in the final phases of the laying period [3].

In turkey breeders a low concentration of vitamin D_3 in the feed (300 IU/kg feed) results in a low egg production and egg weight and eggs with thinner shells. Hatchability percentages were 48% lower with this concentration than with concentrations of 900 and 2700 IU/kg feed [77].

In some studies 1.25 dihydroxycholecalciferol is used. This is the active metabolite of vitamin D_3 (cholecalciferol) and needed in lower concentrations. The use of this vitamin in practice is however limited due to the narrow safety margin between effective and toxic dosages, especially in high calcium diets [1].

8.1.12 Vitamin E / alfa-tocopherol

The requirement mentioned is 10 IU/kg for broilers, 12 IU/kg for turkeys, 5 mg/kg for layers, 10 mg/kg for broiler breeders and 10-25 for turkey breeders[2].

When considering vitamin E requirements it is always important to also take the concentration of selenium in consideration. A lower selenium concentration increases the vitamin E requirement.

Vitamin E is important for many functions: as an antioxidant in blood and on a cellular level, in the maintenance of the integrity of cellular and vascular membranes, as a detoxifier, in fertility and in the immune response, both on a cellular and humoral level [1].

A vitamin E deficiency can present itself in several ways: encephalomalacia, transudative diatheses as a result of endothelium degeneration and muscular dystrophy. The muscular dystrophy can occur in skeletal muscles, the ventriculus and the myocardium. Encephalmolacia is however the most often found symptom and presents itself mostly at the age of 10-20 days. The mortality can increase to ten percent. Clinical signs of an encephalomalacia are incoordination, ataxia, recumbence and cycling motions of the legs. At pathology haemorrhages can be found in the cerebellum and sometimes in the cerebrum, together with malacia [4].

The requirement of vitamin E in broilers selected for lean tissue is approximately 30-45 mg/kg feed when the plasma concentrations are considered. Higher levels (150-400ppm) have not resulted in improved production parameters in chickens and turkeys, but they are associated with other benefits on the field of immunity, resistance to stress and meat quality [1]. Boren & Bond however did find an improvement of production parameters when supplementing 240 mg/kg vitamin E: feed conversion (-2.3%), live weight (+0.7%), viability (+0.1%), downgrading (-34%) and a reduction in the occurrence of inflammatory processes (-61%) and septicaemia-toxaemia (-61%) [1].

In 2014 a review was published by Panda & Cherian in which the effects of vitamin E on chickens were described. It was concluded that vitamin E is important for the maintenance of good health, productivity and reproductivity. Vitamin E is also important for the protection of cells against oxidative stress caused by polyunsaturated fatty acids. The occurrence of oxidative stress is decreased by the activity of lipid peroxidase and an increase in the activity of glutathione peroxidase (GSH0Px). This has been shown for newly hatched broilers at a concentration of 300 IU/kg feed. The amount of vitamin E required however depends on the concentration of fatty acids in the feed. In this review it is also described that the concentration of vitamin E in recently hatched broilers declines rapidly from 100 to 5% in the first nine days of live, which indicates that this vitamin is important during the first days of live. Supplementing these chicks quickly enough is difficult, but the supplementation of breeders also results in an increased concentration of vitamin E in the embryo [78].

Vitamin E is important for the immune reaction of broilers when they are exposed to viruses and bacteria. The antibody response is better in broilers which received feed with an additional 25-50 IU/kg compared to broilers given feed with 10.2 IU [79]. Also Boa-Amponsem *et al*. concluded that

additional vitamin E (300 mg/kg instead of 10 mg/kg feed) resulted in a better immune reaction[80]. Rama Roa *et al*. found positive effects of vitamin E (50-100 mg/kg feed) on the cell mediated immune response and the activity of anti-oxidative enzymes. A concentration of 10 mg/kg feed was not effective in this case [81]. It is believed that the effect of vitamin E on the immune response is based on the function of vitamin E as a lipophilic antioxidant, capable of preventing lipid peroxidation in membranes caused by free radicals. It is also shown that vitamin E promotes phagocytic activity of macrophages. An improved immune reaction is proven in cases of coccidiosis, colibacillosis, listeriosis in turkeys and when chicken were vaccinated against Newcastle disease or infectious bronchitis [1]. Zhu *et al*. showed that supplementing turkeys with 100 or 200 IU vitamin E /kg feed resulted in a decreased incidence of *L.monocytogenes* infections after experimental inoculation. This is related to an increased CD^{4+} and CD^{8+} lymphocytes in the supplemented animals [82]. Also Erf *et al*. have showed that the inclusion of vitamin E (46 or 87 mg/kg feed) in the diet results in an increased percentage of mature CD^{4+} and CD^{8+} T-cells in the thymus and spleen of broilers at the age of seven weeks. Perez-Carbajal et al showed that higher levels of vitamin E are capable of improving the innate immune response, measured as heterophil and monocyte oxidative burst [83]. Considering the central immunoregulatory role of these T-helper cells it would appear that additional vitamin E would be beneficial to the overall immunocompetence of broilers [84].

Vitamin E is also a factor that can be important in coccidiosis; 100 IU/kg instead of 10 IU/kg resulted in a reduction of the growth retardation or mortality caused by *E. tenella*. Also for *E. maxima* infections the addition of higher levels of vitamin E resulted in improved weight gain and reduced amount of leasions and oocyst excretion. In this study no effect could be found in chicken infected with *E.tenella* [31].

Kennedy *et al*. evaluated the economic effect of supplementing broilers with vitamin E (160 mg/kg) and found that the increase in growth and the improvement in feed conversion resulted in a benefit of 8.4% and 2.7% after a deduction of the costs [85].

The effect of vitamin E on the immune system is also evaluated in chickens exposed to heat stress. Vitamin E in a concentration of 125 mg/kg significantly improved the feed conversion rate, but did not influence feed intake or body weight. The primary and secondary antibody responses were however improved in both thermoneutral and heat stress conditions when broilers were supplemented with 125 or 250 mg/kg feed [86]. A positive influence on the prevention of oxidative stress in broilers is also shown specifically during periods of heat stress. In this study 200 mg/kg diet was used [87]. Also in another study the effects of vitamin E on broilers exposed to excessive temperature has been shown: these chicken showed at smaller rise in body temperature and a reduction in mortality [1]. Maini *et al.* showed that vitamin E supplementation (200 ppm) in broilers experiencing heat stress resulted in a reduction of peroxidation of erythrocytes and in tissues [88].

The occurrence of white stripes in the meat of broilers could not be decreased when 15-400 IU of vitamin E was added to the feed [89].

The supplementation of vitamin E (60 mg/kg) in laying hens during periods of heat stress increases the feed intake and egg production. The egg weight is not changed [90]. Also other authors have found an increased egg production and feed intake when 250 mg of vitamin E was supplemented per kg feed. A lower concentration of 65 IU/kg feed however also positively influenced the egg production during longer periods of heat stress. The functioning of the immune system is also improved [34]. Also in Barroeta *et al.* several studies are discussed in which the authors conclude that vitamin E supplementation improves the immune status of the hens and is potentially beneficial during stressful

situation such as high temperatures, transportation, vaccination and molt. Concentrations used are 50-65 IU/kg, 125-300 mg/kg, 250IU and 500 IU [14].

In hens vitamin E is also important for the production. Oxidative destruction of the ovaries may reduce the egg production and there is evidence that vitamin E facilitates the release of vitellogenin, a precursor of yolk, from the liver. Vitamin E is assumed to increase the circulation of compounds necessary for yolk formation [14]. Supplementing hens with vitamin E in a concentration of 50 IU/kg resulted in an increase in egg production from 94.3 to 96.1% [91].

In breeders supplemental vitamin E results in an increase in egg production, especially in the late phase of the laying period and during situations of environmental stress. This effect depends on the breed and age of the animals [92]. An increased quantity of vitamin E in the breeder feed has been shown to increase the concentration in the egg as well as the oxidative stability of the hatching egg and developing embryo [93, 94]. Inadequate vitamin E in the diet of breeders gives rise to the production of eggs with low hatchability and high mortality in the last phase of incubation due to failures related to the circulatory system. Vitamin E is effective in counteracting the negative effects of stress factors such as toxins, anti-nutritional factors (e.g. vicine) or aging on hatchability [3].

Supplementing breeders with vitamin E also results in an improvement of the immune reaction in the progeny: better humoral immunity, more active lymphocytes and increased antibody titres after vaccination against Newcastle disease [1].

Vitamin E is also important for the fertilizing capacity of spermatozoa of breeder males. Vitamin E acts as an antioxidant in the testicles, where it protects the biological membranes from lipid peroxidation during spermatogenesis and in the seminal plasma where it protects against the free radicals which attack the lipids present [95]. Breeder males deficient in vitamin E are less fertile due to the lower quantity and quality of sperm produced. This is especially true for older males [3].

The use of vitamin E in turkey breeders is even more important than in chicken breeders. This can be explained by the fact that the absorption of vitamin E in turkeys is low and the reserve in newly hatched turkeys is lower than those described in chickens [3].

8.1.13 Vitamin K / menadione

According to the NRC the vitamin K requirement of broilers and turkeys is 0.5 and 1.75 mg/kg feed respectively. In laying hens the concentration advised is 0.5 mg/kg. The recommendation for broiler and turkey breeders are respectively 1 and 0.5-1 mg /kg[2].

Vitamin K is essential in the synthesis of prothrombin and factor VII, IX and X of the coagulation cascade. Besides, it is important in different metabolic functions, including the metabolism of calcium. Especially In chicken, vitamin K is essential for the functioning of osteocalcin, a mineral-binding protein which is present in the bones of chicken embryos and in the bone matrix. Osteocalcin is needed for the bone mineralization process [3]. In layers low levels of osteocalcin and bone matrix proteins may impede the mineralization process not only during skeletal development, but also in eggshell formation [96]. In deficient flocks the subcutaneous haemorrhages on the head and beneath the wings can be found. At pathology subserosal haemorrhages are noticed [4].

Birds can synthesize vitamin K in their intestines, but to a limited degree. A prolonged treatment with antimicrobials will eliminate intestinal flora that synthesize this vitamin. The most damaging of these is sulfaquinoxaline. In the case of coccidiosis the vitamin K requirements presumable rise to 8 mg/kg [1].

In laying hens the use of vitamin K can possibly prolong the modelling period of bone formation or inhibit medullary bone loss during the first phases of laying [48]. In a more recent study it was shown that increasing vitamin K levels of 0 to 32 mg/kg at the late stages of production improves performance and bone mineralization, but does not affect eggshell quality [97].

Vitamin K supplementation to birds infected with *E.necatrix* or *E.tenella* infection led to a reduction in coccidiosis associated mortality. There was however no effect on growth rate, blood loss in faces and haematocrit concentrations. When supplemented in chicken with *E. maxima, E. acervulina* or *E. brunetti* infections there was no effect at all [31].

7.2 Minerals & Trace elements

7.2.1 Copper

The copper requirement for laying hens is 5 mg/kg in de first 6 weeks of life and 4 mg/kg for the remainder of the rearing. The requirement for laying hens in production is unknown. For broilers a concentration of 8 mg/kg is advised. In breeders also 8 mg/kg is advised during the first 8 weeks of life. After 8 weeks of life the requirement is 6 mg/kg[2]. The requirement of copper in poultry increases in hens with chronic enteritis [98] or when the animals are exposed to more stress [99]. In the literature there are no recent studies in which the copper requirement of poultry has been investigated.

Copper is essential for the iron transport and metabolism, red blood cell function, the humoral and cellular immune responses [99] and normal bone development [2, 100]. In layers and breeders a sufficient amount of copper is also needed for good fertility rates [100] and egg shell formation [100-102].

A copper deficiency results in anaemia with small red blood cells and low haemoglobin concentrations and possible bone deformations. Copper is also important for the activity of the enzyme needed for the cross-linking of lysine in the protein elastin, which is important for blood vessels. In animals deficient of copper aorta aneurisms and cardiac hypertrophy might occur.

In the field copper is used in animals at risk of gastrointestinal problems. According to Cohen copper can contribute to intestinal health in two ways. First as an antimicrobial agent with activity against *E.coli* and *Salmonella* spp. Secondly, copper would protect the mucosal cells from free radical damage and thereby prevent mucosal damage or promote its recovery [99]. Pang et al showed with in vitro studies that copper can result in higher *Lactobacilli* spp and lower *E.coli* concentrations, but concludes that more research is needed [103]. The EFSA has done a literature search on the effects of copper on the gut microbiota. In low concentrations (<50mg/kg feed) it affects mainly clostridia. Higher concentrations (>200 mg/kg feed) also influence lactobacilli and coliform bacteria [104].

Positive effects on production parameters such as average daily gain and feed conversion rate have also been studied extensively. Most authors found an effect with concentrations of 125-188 ppm, which is higher than the allowed concentrations. Effects on production are mainly seen in 'high microbial environments' [100, 105-107].

7.2.2 Iron

According to the NRC the amount of iron in the broiler feed should be 80 mg/kg. In turkeys the requirement is 80, 60 and 50 mg/kg for respectively the first 4 weeks of life, week 4-16 and week 16 and thereafter. In the rearing of hens the advice is 80 mg/kg in the first 6 weeks and 60 mg/kg until the 20th week in white egg laying strains. For brown egg laying strains the concentrations are respectively 75 and 56 mg/kg. For hens in production 56 mg/kg is advised [2].

An iron deficiency results in anaemia with small red blood cells and a lower amount of haemoglobin.

No literature has been found about the iron requirements of poultry.

7.2.3 Magnesium

The magnesium requirement for broilers and turkeys respectively 600 and 500 mg/kg. For white egg laying strains during rearing the concentration advised are 600, 500 and 400 mg/kg for respectively the first 6 weeks of life, week 6-12 and week 12 and thereafter. For brown egg laying strains these concentrations are respectively 570, 470 and 370 mg/kg. During production the laying hen requirement is 625 mg/kg[2].

Chickens fed diets deficient of magnesium first show a growth retardation and later show a stop in growth and lethargy. When a diet is fed with marginal magnesium concentrations growth is slightly decreased but the animals exhibit lower magnesium concentrations and symptoms of neuromuscular hyperirritability when disturbed. In hens the results of a magnesium deficiency are withdrawal of magnesium from the bone, a decline in egg production and eventually a comatose state and death. In breeders it is important that also the hatchability decreases [2].

In poultry there are several studies done to test the effect of giving more magnesium to chicken. Yang *et al.* recently showed that magnesium (0, 2.5 or 5 mg/kg feed) is important for the prevention of oxidative stress in chicken which experience heat stress [108].

An increase in the concentration of magnesium in the feed from 1.6 to 2.3 or 3 g/kg feed resulted in an improved egg shell quality without negative effect on the egg production [109]. High concentrations of magnesium were also found to be important for the egg shell quality [110].

7.2.4 Manganese

The requirement of manganese for broilers and turkeys is set at 60 mg/kg. In white egg laying pullets the requirement in de first 6 weeks is 60. In the remainder of rearing the requirement is 30. For brown egg laying pullets the requirements are 75 and 56 mg/kg. During production the requirement in laying hens is 25 mg/kg[2].

A manganese deficiency in chicks and poults results in perosis or slipped tendon. Signs of perosis are swelling and fattening of the hock joint with slipping of the Achilles tendon. The tibia and tarsometatarsus may bend near the hock joint and show lateral rotation and shortening and thickening of the long bones of wings and legs can be observed. In laying hens and breeders a manganese deficiency results in a decreased egg production, reduced eggshell strength, poor hatchability and reduced fertility [2, 4].

Manganese has a positive influence on the egg shell quality and bone mineralisation of the tibia in young hens [111]. A deficiency in manganese results in a lower egg production reduced egg shell strength, poor hatchability and reduced fertility [2].

In a reasonable recent study different concentrations of manganese are administered to poultry (23, 53, 83 or 143 mg/kg). There was no difference in body weight, feed conversion rate, mortality or organ weights for the different concentrations [112]. Manganese (104.9 mg/kg) did not have an influence on the metabolism of cholesterol and lipids [113].

7.2.5 Zinc

The minimal zinc requirement is 40 mg/kg for broilers. In turkeys the requirements decline with age: 70 mg/kg for week 0-4, 65 mg/kg for week 4-8, 50 mg/kg for week 8-12 and 40 mg/kg for the weeks 12-

20. In the rearing of pullets for white egg production an amount of 40 mg/kg is advised during the first 6 weeks and an amount of 35 mg/kg for the remainder. In brown egg laying pullets these requirements are respectively 75 and 56 mg/kg. For laying hens during production the advised concentration is 44 mg/kg [2].

According to a recent publication, the average concentration of zinc in broiler diets is 25-45ppm and with a requirement of 40-60 ppm, supplementation is essential. In this article different studies are evaluated and it is concluded that the plasma level of zinc reaches a plateau when the feed contains 45-60 ppm. The bone concentration reaches its plateau at 70 ppm [114].

There is one study in which the optimal amount of zinc for broilers has been studied. For the period between hatching and the age of 21 days, 84 mg/kg feed would be the best concentration [115]. In another study it has been shown that supplemental zinc can improve the anti-oxidant ability and fat metabolism-related enzymes of broilers. This effect was shown for a total concentration of 90 mg/kg feed, of which 60 mg was supplemented compared to the control group (30 mg zinc/kg feed). Higher concentrations of 120 and 180 mg/kg feed did not have additional positive effects. The zinc sources was not relevant for the effects found in this study [116].

Zinc is important for many biochemical functions. A deficiency causes retardation in growth and fraying of the feathers. Bone deformations might involve shortening and thickening of the long bones in the legs and wings and enlargement of the hock joint. In layers and breeders a deficiency in egg production and a reduced hatchability are possible results [2]. In broilers a zinc deficiency is characterized by a lower growth rate and chondrodystrophy [4].

In a review published in 2004 the importance of zinc for poultry is described: it is a cofactor for different enzymes and it is important for cellular immunity, normal growth and maintenance of bone tissue, feathers and appetite [117].

More recently a review has been published about the positive effects of zinc in chicken experiencing heat stress. They concluded that zinc is one of the most important components of the poultry diet during times of heat stress [118].

Georgieva *et al*. studied the effect of zinc on oxidative stress occurring after an infection with *Eimeria acervulina* in broilers. A concentration of 0.17 g zinc per kg feed had a positive influence on the occurrence of oxidative stress [119].

In laying hens zinc supplementation results in an increase in albumen weight, but without any effect on total egg weight or egg shell thickness [120].

7.2.6 Phosphorus

The NRC advises the use of phosphorus (non-phytate) in broilers at a concentration of 0.45% in the first 3 weeks, 0.35% in week 3-6 and 0.30% in broilers older than 6 weeks of age. Also in turkeys the requirements decline with age: 0.6% in week 0-4, 0.5% in week 4-7, 0.42% in week 8-12, 0.38% in week 12-16, 0.32% in week 16-20 and 0.28% in week 20-24. In pullets the advised concentration in feed is 0.40% (week 0-6), 0.35% (week 6-12), 0.30% (week 12-18), and 0.32% (week 18-lay) for white egg laying chicken. In brown egg laying chicken these requirements are the same, except for an advice of 0.35% during the last period. During lay the recommendation is 0.31% [2]. In a very recent study it was concluded that a dietary level of 0.38 % phosphorus (non-phytate) is sufficient for broilers up to 14 days of age [121].

In plant materials phosphor is usually present as phytate phosphorus, which is badly digested by poultry. It is therefore recommended to use other phosphor sources [2].

Phosphorus is mainly known for its function in bone formation, together with calcium and vitamin D_3. A deficiency in any of these nutrients will result in rickets. Another sign of a phosphorus deficiency is poor growth. Additionally, phosphorus is required for the utilization of energy and in structured components of cells such as ATP and phospholipids.

During periods of heat stress, the supplementation of addition phosphorus might be needed; chickens given higher concentrations of phosphorus are more tolerant to high ambient temperatures than those fed ratios with normal phosphorus levels [2].

Wilson *et al*. showed that increasing the phosphorus level from 0.31 to 1.42% did not significantly influence hatchability. The egg production increased when the concentration of inorganic phosphorus is increased to 0.41% but with a higher supply the egg production decreased [122].

2. Pigs

2.1 Vitamins

2.1.1 Beta-carotene

Beta-carotene is a pro-vitamin of vitamin A. Pigs are less well capable of transferring beta-carotene in vitamin than other animals. There is no requirement for dietary beta-carotene to replace vitamin A mentioned in the NRC [123], but in a very old study some authors found that 25 µg per kg bodyweight is needed to compensate completely for vitamin A deficient diets [124]. Of all carotenoids, beta-carotene has the most pro-vitamin A activity [125].

The absorption of beta-carotene in pigs is lower than in other animals [126, 127]. It has also been shown that the small quantities that are absorbed in pigs are converted into vitamin A very quickly [127].

It is doubtful that beta-carotene is required for lymphocyte function, but it may be important for optimum immune function during periods of stress [128]. In two studies from the same author it was shown that beta-carotene is absorbed specifically by lymphocytes [128, 129]. Also Chew et al showed uptake of beta-carotenoids by neutrophils and lymphocytes of pigs and an effect as antioxidants in the subcellular organelles to protect against oxidative damage. The lymphocyte proliferative response against mitogens can be enhanced by beta-carotene [125]. Not all authors find a positive effect of beta-carotene on the immune system [130].

The antioxidant activity of beta-carotene depends on protection against oxygen medicated lipid peroxidation. It has a complementary effect on the usage of antioxidants such as alfa-tocopherol, which inhibits free radical mediated lipid peroxidation, but has no effect against oxygen mediated peroxidation [131].

Beta-carotene use in gestating sows can result in a decrease in embryo mortality and an increase in number of weaned piglets and weaning weight [127, 132]. The effect is possibly partly explained by composition of the uterine fluid; the excretion of retinol into the uterine lumen is thought to be important for early embryonic development. There is however the expectancy that there is an effect of beta-carotene independent of vitamin A. One of these is the role in steroid production, which is also important during early gestation [133]. In vitro studies showed that the progesterone production of pig luteal cells was increased in cells exposed to beta-carotene [134].

It is however suggested that effects on reproduction are mainly seen in sows maintained in operations of low reproductive performance [127, 132]. Tokach et al found two possible reasons for the lack of a significant effect of a beta-carotene injection; production was already very good and only a single injection was given [135].

In another study an effect of parity on beta-carotene injections was found; only in multiparous sows the number of piglets born alive could be increased and the number of piglets born dead decreased [136].

2.1.2 Betaine

Betaine is not an essential vitamin and there is therefore no requirement mentioned in the Nutrient Requirements of Swine [123].

Betaine is an oxidation product of choline and the supplementation of betaine can at least partly replace the choline requirement. When choline deficient diets are supplemented with betaine this will promote growth and prevent liver lipid accumulation [137]. Betaine can however not be converted back to

choline and betaine can only replace choline in its role as methyl donor, not in its essential functions such as synthesis of phospholipids [138].

Betaine can improve the growth rate of pigs, especially in combination with a high protein diet. The betaine supplementation might be more efficient in barrows than in gilts [139]. Some other studies also show beneficial effects of betaine on growth [140]. The effects of betaine on growth can possible be explained by effects on the metabolism if pigs; Schrama et al showed that this can be altered with the supplementation of betaine. The supplemented pigs reduced heat production and energy requirements for maintenance and thereby increased energy retention [141].

Other studies however do not show a significant effect on growth [142-145]. In yet another study conflicting results on the average daily growth were found. This author explains that betaine may have a positive effect on overall proteins status and that the effect of betaine in pig diets depends on the protein and energy status of the feed [146]. Lipinski et al describe that betaine has most effect when added to low energy pig diets [147].

In a study done in feed-restricted pigs it has been shown that the protein: fat ratio increased 19%, indicating that the carcass protein disposition is enhanced at the expense of carcass fat and in part, visceral tissue [143]. The supplementation of betaine in the feed at concentrations of 0.25% improved carcass traits and some aspects of pork quality [148].

In sows the supplementation of betaine can improve reproductive performance and results in a reduced amount of sows returning from oestrus and increased litter sizes in higher parity sows [149]. In another study it was shown that the litter weight at weaning increased and the weaning to oestrus interval decreased in gilts supplemented with betaine. In second parity sows the number of piglets born alive increased, as well as the number of piglets weaned per sow. In this trial betaine was given from 5 days before farrowing to the end of the lactation period [150].

2.1.3 Biotin

The guideline for biotin in piglets is 0.08 mg/kg feed for piglets of 3-5 kg feed and 0.05 mg/kg feed for piglets in the weight of 5 to 120 kg. Based on the diet of growing pigs, the nutrient requirement can however increase to 0.11 mg/kg feed. In gestation and lactating sows the requirement is 0.2 mg/kg feed or 0.4 and 1.1 mg per day respectively. Sexually active boars need 0.2 mg/kg feed or 0.4 mg per day. The use of sulphonamides probably reduces the production of biotin in the intestinal tract, with an increased requirement as a result [123].

There are some studies in which the required amount of biotin is investigated. In a study by Kopinsky *et al*. the administration of 100 µg/kg was high enough to prevent deficiency signs from occurring [151]. In another study deficiencies occurred when the concentrations in the feed were below 50 µg and maximum tissue concentrations were reached with a concentration of 100 µg/kg feed [152]. Hamilton *et al*. showed that when the feed contained 22 mg of biotin per kg, the administration of extra biotin was no longer beneficial [153].

Biotin is prevalent in pig feed, but the biological availability can vary greatly between different sources [154]. Biotin can be produced by bacteria in the intestines of pigs and therefore it was long thought that supplementation was not needed. A deficiency can however occur in the field [155].

Biotin is a cofactor of various enzymes and plays an important role in lipid, carbohydrate, protein and nucleic acid synthesis. A deficiency can therefore result in very asymptomatic signs such as a reduced growth rate, poor feed conversions, a reduction in reproductive efficiency etc. [127]. More specific signs of a deficiency are related to the skin and horn. A deficiency of biotin can result in problems with

the skin and the quality of the claws [127, 155]. Effects on the skin are shown in several studies [151, 152, 155]. A deficiency results in a dermatitis, alopecia and depigmentation of the skin [156].

Supplementation with one mg biotin per kg feed results in a change in the structure of the epidermis of the claws; there was an increase in density of the horn [157]. Webb *et al*. also showed that 1 mg of biotin per kg feed resulted in harder horn which can stand more pressure. This could be an explanation for the decreased occurrence of lameness [158]. In the most recent study about this subject Fritsche *et al*. showed that biotin is important for the claws and that a deficiency results in weak, brittle and sometimes necrotic horn. They however also showed that supplementing biotin is not only beneficial in deficient animals [156]. In a study in the Netherlands in which field data were obtained it was shown that adding biotin to the feed in a systemic way resulted in a reduction of sows culled as a result of lameness by fifty percent [127].
The NRC also mentions that biotin supplementation in sows can result in an improvement in hoof hardness and compression, compressive strength, and the condition of skin and hair coat, as well a reduction in hoof cracks and footpad laesions. They are however also studies in which this could not be shown [123]. In a study done in sows it was shown that biotin supplemented at a dosage of 440 µg/kg feed resulted in a lower incidence of hoof leasions, heel cracks, heel-horn junction cracks, side-wall horn cracks and white-line horn cracks [159]. Also in another study a significant reduction in heel erosions, white-line disease, heel bruising and severity of the laesions was noticed [160]. In a Dutch field trial it was proven that supplementing biotin in sows feeds resulted in a decrease in claw leasions in gilts by 28%. In the replacement stock the lesions score decreased with 52%. As a result the culling rate caused by lameness decreased from 25% to 14%. Also the culling rate as a result of insufficient production could be decreased from 11% to 4% [161]. In another study the culling rate of sows could not be reduced, despite a significant effect of biotin supplementation on claw laesions when biotin was supplemented in an early stage of development [162]. Misir and Blair evaluated the effect of 100-400 µg/kg feed and concluded that the higher the concentration, the quicker the effect on incidence of foot laesions and skin problems [163].

Old studies suggest that biotin does not affect the growth of piglets. Kopinsky *et al*.[151] showed that 100 µg/kg feed did not increase the feed efficacy and Kornegay *et al*. [164] showed that supplementing the feed with 220, 440 or 880 mg of biotin/kg feed had no influence on daily weight gain. In a more recent study it is however suggested that when biotin is added to the feed (440 µg, basal feed: 220 µg) the average daily gain (ADG) increases [165]. Also Martelli *et al*. found an increased ADG when pigs were supplemented with 150 or 300 ppb [166].

There is a study in which it is shown that the feed utilization increases with 7.7, 8.4 and 8.2% after the supplementation of 200, 300 and 400 ppb respectively. Also the carcass yield, yield of hams and digestibility and tenderness of the meat improved. The concentration unsaturated fatty acids in back fat decreased [167]. A decrease of unsaturated acids and an increase in unsaturated acids in the meat was found by Martelli *et al*. [166].

The immune system can be supported with biotin: supplementing the feed with 220 or 440 mg biotin/kg resulted in a slightly increased immune response to sheep red blood cells. Higher concentrations of 880 mg/kg feed however seemed to depress the response. More research is thus needed [164].

Supplementing sows with biotin (200-500 µg/kg feed) resulted in more piglets being born and a higher weight at weaning [127]. Also the NRC mentions that supplementing sows with 0.33 mg biotin per kg feed resulted in an increased number of pigs weaned per sow [123]. Lewis *et al* found that the litter size at birth was not influenced by biotin supplementation (330 µg/kg feed). The number of the number of piglets after three weeks of lactation was increased in the supplemented sows [168]. Penny *et al* did

however find an increase in litter size at birth [169]. Brooks *et al* found an increase in litter size at birth, but this difference was only statistically significant in second parity sows [170]. In another study supplementing 0.55 ppm in the feed was sufficient to increase the number of piglets per litter [171]. Bryant *et al* showed only a tendency for an increase in total and live number of pigs farrowed from sow supplemented with 440 µg biotin per kg feed. These sows did however have an increased conception rate at first oestrus postpartum and a reduced interval from weaning to oestrus [172]. It has also been shown that supplementing biotin to the sow's diet (350 µg/kg feed) results in a shorter interval between weaning and oestrus and conceiving and a decreased number of animals that need treatment for anoestrus [173]. In a study by Watkins *et al* the effects on reproduction could not be reproduced [174].

2.1.4 Choline

According to the NRC the requirement of piglets is 0.3-0.6 mg/kg feed. The requirement for pregnant and lactating sows is respectively 2.3 and 5.3 mg/kg feed. The requirement however depends on the concentration of methionine in the feed. This amino-acid is a precursor for choline and enables pigs to produce choline themselves. A deficiency of choline leads to a decrease in growth rate, a bad fur and a low haematocrit[123].

In research done by Russet *et al*. different concentrations of choline in the feed have been studied and the requirement is maximally 520 mg/kg feed, but more research is needed to specify this further [175].

The effect of choline on growth is questionable. The NRC refers to some old studies in which very high concentrations of choline (1000-1646 ppm) are used to increase the growth rate and improve the feed conversion[123]. In an article from 1986 seven studies are described in which choline is added to the diet. Growth seemed to decrease slightly with a concentration of 6000 ppm. Adding 2000 ppm to the feed for a long period of time also decreased growth, but did not influence the feed conversion rate [176]. Russet *et al*. found varying effects of choline on the growth of pigs [175].

The supplementation of 500 mg/kg feed to sows did not influence the survival rate of piglets, milk production or carcass quality [177].

2.1.5 Folic acid

The requirement in piglets according to the NCR is 0.3 mg/kg feed. The requirement for sows and boars is 1.3 mg folacin per kg feed. A treatment with sulphonamides will increase the requirement of folic acid because it will decrease the intestinal production of this vitamin [123].

Folic acid is especially important in tissues active in protein synthesis and cell division. It participates in the increase of nuclei in muscle cells [127]. A deficiency will result in a growth retardation, a fading hair colour, anaemia, leukopenia, thrombocytopenia, a decreased haematocrit and bone marrow hyperplasia [123, 127].

Folic acid is also synthesised in the intestinal tract, but Asrar & O'Conner predict that a maximum of 18% of the dietary folate requirement for the piglet could be met by folate absorption in the large intestine [178].

In the NRC guidelines a study is described in which adding 11 mg/kg feed had no influence on the growth of piglets (age 17-27 days), but in another study 0.5 mg/kg feed was effective in improving the growth rate[123].

NRC increased the requirements of sows based on several studies that conclude that folacin is needed for optimal production of sows [123]. Lindemann evaluated several studies and found a consistent increase in litter size when folic acid was supplemented during gestation. This seemed to be a result of

improved embryo or foetal survival [179]. This effect was also found by Thaler *et al* [180] and Lindemann en Kornegay [181].

Folic acid supplementation to sows in concentrations of 12.5-100 mg per kg feed also resulted in an increased milk production by the sow. The composition of the milk also changed, resulting in increased weigh gain of the piglets[182] .

Harper *et al* however concluded that folic acid supplementation did not result in an improvement on reproductive and lactation performance, despite an increase in in serum folate concentrations [183].

2.1.6 Niacinamide

The NRC requirements mention information about niacin, not about niacinamide. For piglets of 3-5 kg bodyweight the requirement is 20 mg/kg feed, but it increases to 15 mg/kg for 5-10 kg bw and to 12.5 mg/kg for 10-20 kg bw. Thereafter the requirement decreases; 10 mg /kg feed for 20-50 kg bw and 7 for pigs heavier than 50 kg. For gestating and lactating sows and boars the advised amount of niacinamide in feed is 10 mg/kg, but no studies are mentioned as reference for the determination of this value [123].

Niacinamide is an amide of niacin and both possess the same biological activity.

A niacin deficiency results in growth retardation, anorexia, vomiting, a dry skin, dermatitis, a rough coat, alopecia, diarrhea, mucosal ulcerations, ulcerative gastritis, inflammation and necrosis of the caecum and colon and anaemia [123, 127]. Burroughs *et al* reported poor appetite, slow growth, roughened hair coat, diarrhea, anaemia and disturbances in the large bowel in deficient pigs. Deficiency symptoms could be prevented in these animals by supplementing the feed with 60 mg of niacin per kg feed [184].

Niacinamide is found in niacinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These enzymes are important for the oxidation reduction reactions and are involved in many biochemical processes, especially those linked to energy supply to the cell [127].

Niacin has been proven to increase the nitrogen utilization, nitrogen digestion and the feed intake in pigs when administered at an amount of 14 mg/kg feed. These animals also had a lower incidence of diarrhea than the control animals. Supplementing 6 or 10 mg/kg feed had no influence [185]. In an earlier study from the same authors there was however no effect of niacin (1-81 ppm) on growth, feed conversions rate, the condition of the skin and hairs and the incidence of cracks on the hoofs [186]. Real *et al* showed that the gain to feed ratio improved when pigs were fed diets supplemented with 13 to 55 mg niacin per kg feed [187]. In a study described in the NRC guidelines, there was no effect of niacin on growth rate or the accumulation of protein or fat when the concentration was three times as high as the NRC requirement[123].

Ivers *et al* did not find beneficial effects of supplementing niacin in 33 mg/kg feed to a corn-soybean meal in sows [188].

2.1.7 Vitamin A / retinol

According to the NRC the feed should contain 2200 IU/kg feed for piglets up to 10 kg, 1750 for piglets up to 20 kg. During fattening and finishing the recommendation is 1300 IU/kg [123].The vitamin A requirement of piglets at the age of 1-8 weeks is 800 IU/lb of feed (360 IU / kg of feed) [189]. To obtain a sufficient concentration of vitamin A in the liver, the minimal amount of vitamin A in the feed should be 2000 IU/kg feed for weaned piglets and 800 IU/kg of feed for fattening pigs [190].

The requirement for sows during gestation and sexually active boars is 4000 IU per kg feed. For lactating sows the requirement is 2000 IU per kg feed [123].

A vitamin A deficiency results in growth retardation [123, 190], incoordination, paralysis posterior and blindness. Besides, vitamin A is also important for vision and the formation, protection and secretory activity of the skin and mucosa. It is also believed to be important for the immune system; suboptimal levels of vitamin A could result in a decreased antibody production and a lower number of lymphocytes [127]. However, when there is no deficiency, the administration of an additional amount of vitamin A does not seem to increase the production parameters [191, 192]. Growth and other production parameters could not be stimulated in pigs when the basal ratio of vitamin A in the feed was already 7500 IU per kg feed [193].

When piglets are born with a vitamin A deficiency because the sow was deficient in vitamin A this can result in a lower immune response when these piglets are exposed to a rotavirus. Also during an endotoxaemia vitamin A seems to be important. Injecting this vitamin to piglets improved the cardiac and pulmonary functioning and decreased the concentration of endotoxins [194, 195]. Pederson *et al* proved that a vitamin A deficiency in piglets can alter the functional integrity of the mucosal intestinal epithelium [196].Parenteral supplementation of high doses of vitamin A in young sows at weaning and breeding has resulted in an improvement of the number of pigs born and weaned per litter, indicating that vitamin A requirements for maximal performance may vary with age [197]. In another study however, a single injection with vitamin A was incapable of increasing in litter size in sows [198]. Whaley *et al* showed that vitamin A can alter the development of oocytes and embryos in pigs. The percentage of advanced stages was increased and the variation in stages was decreased [199]. Vitamin A supplementation in sows enhances the liver vitamin A concentration in the offspring; larger one time doses of 2.1 mmol are not more effective than smaller doses of 1.05 mmol [200]. Parental supplementation of β-carotene to sows can improve embryonic survival, possible as a result of an increase in uterine-specific protein secretion. In this study oral supplementation with β-carotene did not have the same result, possible due to poor absorption. Oral administration of vitamin A was not evaluated [123]. β-carotene parenteral or oral supplementation to primiparous sows does not affect production parameter, but in subsequent litters farrowed by multiparous sows, more piglets were born alive and fewer were borne dead [136].

Vitamin A is also important for the sexual activity of boars. Vitamin A is perhaps important for cell membranes and lysosome stability [201].

The tolerance of vitamin A has been determined by Blair *et al* in weanling piglets. They received up to 100 times the NRC requirement. Special attention was paid to the occurrence of osteochondrosis, but the no toxicities were reported [202].

2.1.8 Vitamin B_1 / thiamine

According to the NRC the requirement is 1.5 mg/kg feed for piglets weighing 3-5 kg and 1 mg/kg feed for pigs up to 120 kg bodyweight [123]. The same thiamine requirement of piglets was shown in 1950 (1.5 mg/kg feed), while deficiencies were noticed when the concentration decreased below 1 mg/kg feed [203]. In sows and boars the thiamine requirement is 1 mg/kg feed. [123].

Thiamine is important for the formation of nucleotides and valine and is important for the physiology of the nervous system, probably through its involvement in the formation of neurotransmitters, the transport of sodium and for its role in obtaining energy by the neuron [127]. Pigs with a deficiency of thiamine show a decreased appetite, retarded growth, a lower body temperature, a lower heart rate and

occasionally vomiting. Also abnormalities to the heart can be found such as hypertrophy, a degeneration of the myocard and sudden death [123].

Supplementing the feed with 3.3 mg/kg feed [204] or 200-720% of the recommended requirement [205] has no influence on the daily feed intake or daily growth of piglets in the first two weeks after weaning.

2.1.9 Vitamin B_2 / riboflavin

The requirement according to the NRC is 4 mg/kg feed for 3-5 kg bw, 3.5 mg/kg feed for 5-10 kg bw, 3 mg/kg feed for 10-20 kg bw, 2.5 mg/kg feed for 20-50 kg bw and 2 mg/kg feed for higher bodyweights [123]. There are also some studies in which the minimal requirement of riboflavin in piglets has been studied. In old studies a requirement of 3 mg/kg feed [206] or 1.5-2 mg/kg feed [207] is described. Mitchell *et al*. showed that the riboflavin requirement depends on the environmental temperature; the requirement is 1.2 mg/kg feed at a temperature of 85˚F and 2.3 mg/kg feed at a temperature of 42˚F [208].

The NRC guidelines report requirements of 3.75 mg/kg feed for both pregnant and lactating sows[123]. In lactating sows the requirements of riboflavin is 16.3 mg per day [209] or 1.25 mg/kg feed [210]. Sows that did not ingest enough riboflavin during pregnancy (0.77 mg/kg) had litters with a lower litter weight and a higher mortality [211].

Clinical signs of a deficiency are usually unspecific but involve tissues and functions that depend on carbohydrates for their energy requirement: epithelial and nervous tissues and functions related to reproduction [127]. The following clinical signs have been described in animals with a severe riboflavin deficiency: increased concentration of granulocytes, impaired immune response, liver pathologies, kidney deformations, fatty liver syndrome, degeneration of the ovaria, degeneration of the myelin sheath [123] and inflammation of the anal mucosa [127]. There is also a negative effect on reproduction: anoestrus, embryonic death and reabsorption, birth of weak piglets which die in the first 48 hours, oedema in piglets, premature birth (up to two weeks) and litters without hair [212, 213].

Some authors have found that including higher quantities of riboflavin (60-160 mg/day) in the early stages of gestation had a positive effect on the number of piglets born [213, 214], specifically in farms with poor reproductive performance [127].

In piglets with a weight of 10-27 kg including higher levels of riboflavin (175-330% of NRC requirements) has resulted in an increased growth rate: 6 g/piglet/day/ riboflavin included in the feed [215].

2.1.10 Vitamin B_5 / D-panthenol

The NRC uses a requirement of 12 mg/kg for piglets with a bodyweight of 3-5 kg. The minimum requirement reduces for weights up to 50 kg and the recommendation for piglets heavier than 50 kg is 7 mg/kg feed. In sows and sexually active boars the requirement is 12 mg/kg feed [123].

There are some really old studies concerning the nutrient requirement of panthenol in piglets: Stothers *et al*. [216] found a requirement of 12.5 mg /kg DM (milk) and Palm *et al*. [217] found that the requirement is lower than 13.2 mg/kg feed, but did not find an exact number. Groesbeck found that the growth of fattening pigs could not be increased when the diets already contained 25.7 mg pantothenic acid per kg feed [218].

A deficiency of panthenol results in severe diarrhea and at a later stage incoordination [216]. In piglets fed a deficient diet ataxia occurred as a result of chromatolysis in the afferent neurons [219]. According to the NRC additional deficiency sings are: growth retardation, anorexia, a dry skin, rough hair,

alopecia, a decreased immune reaction and abnormal gait [123]. A slower and less efficient growth was also reported by Harmon *et al* in pigs deficient of vitamin B_5. Deficient pigs also showed lower antibody titres. After supplementation all parameters were equal to those of the control animals [220]. In gestating and lactating sows fatty liver has been observed as well as enlargement of the adrenal glands, haemorrhages, rectal congestion, atrophy of the ovaries and reduced oestrogen and progesterone synthesis, leading to uterine atrophy [127]. Poor reproductive performance has also been shown in diets with a pantothenic acid concentration lower than 5.9 mg / kg feed. Other authors however reported normal reproductive performance at this level [123].
Post mortem laesions that can be found are: oedema and necrosis of the intestinal mucosa, a fibrotic submucosa, loss of the myelin sheath and degeneration of dorsal ganglion cells [123].

2.1.11 Vitamin B_6 / pyridoxine

According to the NRC the requirement is 2, 1.5 and 1 mg/kg feed for pigs weighing 3-5, 5-20 and >20 kg respectively[123]. The requirement of pyridoxine in recently weaned piglets is supposedly high. The abrupt change in feed results in an abrupt change of amino acids which have to be modified. The amount of pyridoxine in the milk is low and can barely meet half the requirement. Supplementing piglets at this age with 7.7 mg/kg is sufficient to avoid the appearance of deficiency symptoms but not to raise the pyidoxal-5-phosphate concentration in erythrocytes to within the optimum range, suggesting that an even higher supply is necessary [127]. Miller *et al*. showed that 0.5 mg pyridoxine per kg feed was enough when growth and feed intake were evaluated. However, when blood parameters (haemoglobin, red blood cells and lymphocytes) were evaluated, at least 0.75 mg/kg feed had to be used and the minimal requirement increased to 0.75-1 mg/kg feed when the excretion pattern of the animals was taken into consideration[221].

Vitamin B_6 requirements in sows are 1 mg per kg feed. Sows fed deficient diets showed an increase in litter size when this vitamin was added at a concentration of 1 mg/kg feed but higher concentrations were not effective in further improving production rates. For sexually active boars it is recommended to include 1.9 mg of pyridoxine per kg pf feed [123].

Vitamin B_6 has a crucial role in the nervous system, specifically in neurotransmitters [123]. It is also a coenzyme of over 50 enzymes which makes it essential for amino acid metabolism [127]. A deficiency will result in a decreased feed intake and growth. Severe deficiencies are associated with exudate around the eyes, convulsions, ataxia, coma and death. Post-mortem examinations reveal degeneration of the myelin sheath, anaemia and fatty liver syndrome [123]. The capacity to utilize protein will also decrease significantly, resulting in a marked drop in nitrogen balance [127].

It has been shown that an additional 3.3 mg pyridoxine per kg feed (piglets 0-14 days of age) results in a better feed intake and increased growth rate [204]. Also in an older study the beneficial effect of pyridoxine (0.03, 0.3 and 3 mg/kg feed) on feed intake and growth has been proven [222]. In pigs fed a supplement with pyridoxine showed increased growth, nitrogen retention, blood packed cell volume and haemoglobin than animals receiving only the control diet [223].

2.1.12 Vitamin B_{12} / cyanocobalamin

According to the NRC the requirement is 20 µg/kg feed for piglets weighing 3-5 kg, 17.5 µg/kg between 5 and 10 kg, 15 µg/kg between 10 and 20 kg, 10 µg/kg for 20-50 kg and 5 µg/kg for pigs weighing more than 50 kg [123]. In an old study Anderson & Hogan found a requirement of 0.26 mg/kg bodyweight or 1.5 mg/kg feed [224]. In adult animals the vitamin B_{12} requirement is 15 mg/kg feed [123].

Signs of a deficiency include growth retardation [123, 127, 225], a decreased feed intake, bad hair growth, dermatitis, hypersensitivity and incoordination of the hind legs. Laboratory tests might reveal anaemia, lymphocytopenia, a high concentration of neutrophils [123, 127] and increased mortality [127]. A deficiency of vitamin B_{12} is most prevalent in young animals. Adult animals can synthesize vitamin B_{12} in their intestines, providing there is enough cobalt in the feed [123, 127].

Bodyweight of 100 pound pigs could not be increased with the addition of vitamin B_{12} alone or in combination with cobalt [226] in one study, but vitamin B supplementation at a rate of 4-6 mg per kg feed resulted in increased growth rate and improved feed conversion rates [227]. Richardson proved that vitamin B_{12} supplementation resulted in a slight improvement in daily gain, but in the presence of antibiotics the improvement in daily weight gain was highly significant [228].

Reproductive performance of sows has been improved with the addition of 11 to 1100 mg of vitamin B_{12} per kg feed [123].

2.1.13 Vitamin C / ascorbic acid

The NRC guidelines do not include vitamin C because this vitamin can be synthesized endogenously. It is however known that the production cannot meet the requirement in specific situations [123, 127]. Situations in which additional vitamin C might be required are pathological diseases (e.g. parasitic illness, infections, tumours, etc.) and stress, particularly heat stress [127]. Also Mahan *et al* concluded that supplementing vitamin C in pig during stressful periods (e.g. heat stress, weaning) can promote pig performance. Routinely supplementing vitamin C did however not enhance pig performance [229]. In a very old study it was also shown that piglets up to one week of age either have a very high vitamin C requirement or that they are incapable of producing vitamin C at this age [230].

In one study the supplementation of 500-1000 mg/kg feed did not result in an improvement of bone quality or change the metabolism of calcium and phosphate in pigs [231], which suggest that the requirement is lower than 500 mg/kg feed.

Vitamin C is an antioxidant and is important for the production of collagen which is important for cartilage and bone tissue [123, 127]. A deficiency can result in collagen abnormalities but also the occurrence of petechia [123]. It has also been linked to the process of wound healing, adrenaline and anti-inflammatory steroid synthesis, the phagocytic function of leukocytes and the production of antibodies, besides being a natural inhibitor of nitrosamines which are potent carcinogenic agents [123, 127]. Vitamin C is also important in the reduction of cellular ferritin iron for transport to the body fluids. In sows vitamin C might be related to insufficient collagen cross-linking because of reduced hydroxylation of lysine, resulting in osteochondrosis [123]. Yen *et a*l found that vitamin C supplementation to piglets between four and five weeks of age was needed for maximum weight gain and that the beneficial effect of this supplementation is related to iron metabolism [232].

The study of the effect of vitamin C on growth has revealed varying results, but most studies found no significant effect on growth [123, 233, 234]. In Isabel *et al.* some studies are described in which there was an increase in growth [127]. There is no influence on feed intake, feed conversion rate [234] or mortality [123]. Vitamin C supplementation to sows resulted in increased bodyweight in progeny at the age of three weeks. The problem of navel bleeding prevalent in this herd also ceased quickly after the administration of vitamin C to sows at 1 gram per day was started. In subsequent studies no effect on growth could be found when sows were supplemented with 1-10 gram per day [123]. Also Yen *et al* did not find any beneficial gestational or lactational effects of supplementing sows or gilts from 108 days of gestation to seven days in lactation [235].

The effect of supplementing vitamin C 48 hours before slaughter (1000-2000 mg/liter water) on meat quality has been studied, but no effect could be shown [236].

There seems to be a beneficial effect of vitamin C on the immune system, specifically on the immunoglobulin concentration [234], but more research is needed to confirm this.

2.1.14 Vitamin D_3 / cholecalciferol

The vitamin D_3 requirement of piglets is 220 IU/kg feed, 200 IU/kg feed for pigs weighing 10-20 kg and 150 IU/kg for the remainder of the fattening period. In sows and sexually active boars the vitamin D_3 requirement equals 200 IU per kg feed, but unfortunately this is based on limited data only [123]. In a study published in 2010 it was shown that the vitamin D requirement of sows should be 1400 IU per kg feed [237], which is significantly higher, than the NRC requirement.

A deficiency of vitamin D_3 results in a decreased growth rate [127] and decreased absorption of calcium and phosphor resulting in calcification problems. In young piglets rickettsia can be formed, in adult animals osteomalacia is the result [123, 127]. In very severe deficiencies tetany can occur as a result of a calcium deficiency [123]. The absorption of magnesium from the gastro-intestinal tract can also be increased with the supplementation of vitamin D in the diet [238].

Supplementation of vitamin D3 in concentrations above the basal concentrations (1378 IU/kg nursery feed) does not result in an increased growth or feed conversion rate in in pre-weaning and nursery pigs [239].

In recent studies it has been shown that supplementing additional vitamin D_3 (6-110 IU/kg) can benefit the skeleton: it contributes to a normal endochondral ossification, it inhibits progression of osteochondrosis and regenerates destroyed cartilage [240]. In an older study it was also shown that vitamin D_3 increases mineralisation of bone and dentine [241]. Another study however found that very high concentrations were not effective in improving bone mineralization when the basal feed already contained vitamin D_3 at a concentration of 1378 IU/kg [239].

A recent study indicates that vitamin D_3 also has a positive immunomodulatory role in pigs; increased concentrations of vitamin D_3 results in an increase in leukocyte cell counts and a positive modulation of leukocyte survival and phagocytic capacity across blood and bronchoalveolar compartments, highlighting the potential impact on systemic and mucosal antimicrobial responses [242]. Zhao *et al* recently showed that supplementing vitamin D can mitigate the negative effects of a porcine rotavirus challenge, such as a decrease in weight gain, feed intake, villus length and faecal consistency [243].

Piglets are born with low vitamin D plasma concentrations and the sow milk also contains little vitamin D. Supplementing sows with 50 µg instead of 5 µg per kg feed results in higher plasma concentrations in the progeny. After weaning the average daily feed intake was greater and growth performance tended to increase in the piglets from supplemented sows [244]. Goff et al found that supplementing sows parentally with vitamin D before parturition is an effective mean of supplementing young piglets via the placenta and milk, even though no significant effects were seen on plasma vitamin D status of the sow or piglet [245]. On the other hand, Lauridsen found that little vitamin D is transferred to the progeny, irrespective of the dietary dose and form of vitamin D provided to the sows [237].

Supplementing vitamin D_3 in extremely high concentrations can also have negative effects: cell degeneration and fibrinomuscular thickening of the arterial wall [239, 246-248]. This occurred with concentrations of 62,500 IE/kg feed for a period of three months [248] or 220,000 IE/kg feed (unknown period) [247]. Vitamin D toxicosis can be accompanied by anorexia, polydipsia and polyuria. After two weeks a rough hair coat and impaired growth rates can be observed [249]. Long discovered an

haemorrhagic gastritis, a diffuse interstitial pneumoniae and myocardial degeneration in pigs with a vitamin D toxicosis [250]. Feeding pigs with 825000 IU vitamin D per kg feed resulted in hypercalcaemia, anorexia, weakness, a rough hair coat and laboured breeding. The hypercalcaemia was caused directly by osteonecrosis and nog by an increased absorption rate [251]. Toxicity signs were found in another study when 25000 IU of vitamin D was supplemented to piglets per day [252]. Blaire examined the effect of feeding levels of up to 100 times the recommendation of 200 IU/kg feed from the NRC. After four weeks no effects was noticed on growth rate, feed intake or feed conversion rate [253].

2.1.15 Vitamin E / alfa-tocopherol

The vitamin E requirement is 16 IU/kg feed for piglets <10 kg and 11 IU/kg feed for piglets of 10-120 kg bodyweight. The requirement however depends on the concentration of selenium and antioxidants in the feed. Vitamin E is easily degraded in feed by warmth, moisture, fat or trace elements which make it difficult to determine the actual amount of vitamin E in the feed ingested by the animals [123]. In the literature requirements of 30 mg/kg feed [254] and 80-100 IU /kg feed [255] have been suggested. The relation between selenium and vitamin E has also been demonstrated: the vitamin E requirement is 15 IU when the concentration of selenium is 135 mg/kg but increases to 20-30 IU when the selenium concentration in the feed is 0.1 mg [256]. In a study in Norway it was shown that the vitamin E supplementation to piglets and weanling pigs was still suboptimal, but that the two nutrients can partly compensate for each other during the weaning period [257].

The vitamin E requirement of sows and sexually active boars is 44 IU per kg feed [123]. The vitamin E status of young pigs also depends on the supplementation to sows. Increased concentrations during gestation results in piglets born with more vitamin E. increased concentrations during lactation results in increased uptake via the milk [258].

The vitamin E requirement possible also depends on the vitamin A level of the feed. High vitamin A levels produce low fat and liver α-tocopherol levels [259]. In another, but quit old, study it was however concluded that vitamin A level in the feed does not influence tissue α-tocopherol levels in growing-finishing swine [260].

A vitamin E deficiency results in different clinical signs: degeneration of bones and muscles, thrombosis, parakeratosis of the stomach, stomach ulcers, anaemia, liver necrosis, yellowing of the adipose tissue and sudden death [123, 127]. The occurrence of mulberry heart disease is also linked to the vitamin E concentration in the liver [261]. A vitamin E deficiency can also influence the immune system, probably by a suppression of the mitogen-induced lymphocyte proliferation [262].

An increased growth rate and improved feed conversion rate could not be induced by vitamin E with 22 IU /kg feed [123] or 220 IU/kg feed [263].

Vitamin E has also been related to the immune response of pigs [123]. Wuryastuti *et al* showed that vitamin E deficiencies depressed the responses of lymphocytes of and polymorphonuclear cells [264]. Supplementing pigs with 100 instead of 20 IU per kg feed resulted in an increase in immunoglobulin response with 2-3 times after an injection with an *E.coli* bacterin [265]. Peplowski *et al.* also found a positive effect when supplementing pigs with 220 IU of vitamin E in the feed [266]. In a more recent study the effect of vitamin E on the immune system has also been shown [267]. Mudron however could not find an effect on several indicators of the immune system when vitamin E was supplemented when 100 or 200 mg of vitamin E was added to the diet [268]. Hayek *et al* showed that an injection with vitamin E or selenium increased IgM concentrations in colostrum, but this was only significant after an injection with selenium. Both treatments resulted in significant increased IgM concentrations in the

piglets serum [269]. Nemec *et al* could not find any influence of vitamin E supplementation between 22 to 88 IU vitamin E per kg feed during gestation and 55 to 220 IU/kg feed during lactation and the IgG, IgM and IgA concentrations in colostrum and milk [270]. Bonette *et al* report that vitamin E supplementation of up to 550 IU per kg feed does increase the serum levels of vitamin E but does not influence the humoral or cellular response [271].

Agger *et al.* showed that adding 500 IU of vitamin E per 200 kg of bodyweight does not only result in an increased immune reaction, but results in clinical improvement. On a farm with diarrhea problems supplementation with this vitamin resulted in less severe diarrhea and a lower mortality rate [272]. In another study under field conditions vitamin E at a concentration of 20-80 IU/kg feed resulted in better control of diseases problems with post weaning diarrhea as example. On this farm, this resulted in a decrease in the amounts of antimicrobials used [273]. In another study the clinical signs caused by dysentery could be reduced with the use of vitamin E. When this vitamin was however combined with selenium, it also resulted in an increased growth rate [274]. Administering a diet rich in vitamin E does not result in mitigation of the morbidity effects of porcine reproductive and respiratory syndrome virus infections in nursery pigs [275].

In sows vitamin E might be important in the mastitis metritis agalactia (MMA) syndrome. The content of vitamin E in sow colostrum and milk depend on the content of the sow's diet [123]. Babinszky *et al* showed that vitamin E supplemented to sows results in an increased vitamin E concentration in the progeny and also improved the immune response in these piglets [276]. Mahan found that increasing the vitamin E supplementation to sows results in larger litter sizes at seven days post-partum. The piglets also had higher plasma vitamin E levels. Evidence of a vitamin E deficiency occurred at 28 days post weaning in sows given a diet with 16 IU of vitamin E. Higher levels are thus justified for reproducing sows [277]. Mahan later found a decreased incidence of mastitis, metritis and agalactia when sows were supplemented with vitamin E in concentrations up to 66 IU / kg feed. Also the number of piglets born was increased significantly. There was a tendency for an increased number of piglets born alive. The concentration of vitamin E in the milk was also higher than in the control group. Based on this information it is concluded that the recommendation from the NRC might be too low [278].

The effect of using vitamin E as an antioxidant to stimulate the quality of the carcass has been extensively studied. The occurrence of polyunsaturated fatty acids in the meat can fasten the degradation process. Antioxidants such as vitamin E can delay or decrease the oxidation process by protecting biological membranes [279-289]. The amount of vitamin E used in different studies to acquire this effect range from 25 to 300 mg/kg feed and in one study even to 500-1000 mg/kg feed. The recommended period of supplementation is six weeks [287]. When the concentration of polyunsaturated fatty acids increases in the feed, more vitamin E is needed [290]. Supplementing vitamin E in combination with selenium has an even better effect on the oxidative status of the meat [285]. Apart from the quality of the meat, also the colour depends on the concentration of vitamin E. Supplementation of animal rations with different quantities brought about better colour stability and a lesser reduction in "A" value (directly linked to the colour red) than were found in not supplemented animals [127]. In a review from 2011 it was concluded that vitamin E is effective in improving lipid and colour stability of meat. Models are provided to determine the amount of supplemental vitamin E advised [291].

2.1.16 Vitamin K /menadione

The NRC advices the use of vitamin K in feed at a concentration of 0.5 mg/kg feed for pigs of all ages, including sows and boars. Vitamin K is also produced in the intestines of pigs [123] and the

administration of antimicrobials might reduce the intestinal production of this vitamin resulting in a deficiency [127].

Vitamin K is essential for the synthesis of prothrombin and factor VII, IX and X of the coagulation cascade. It is also important for the metabolism of calcium [123, 127]. The main symptoms of vitamin K deficiency are the increase in blood coagulation time and anaemia [127]. Also in the field a prolonged prothrombin time, hypersensitivity, anaemia, anorexia and weakness have been described as a result of vitamin K deficiency [292].

2.1 Minerals & Trace elements

2.1.1 Copper

The nutrient requirements are 6 ppm for piglets up to 10 kg bw, -5 ppm for piglets of 10-20 kg bw, 4 mg/kg for a bw of 20-50 kg, 3.5 mg/kg for a weight of 50-80 kg and 3 mg/kg with a bw of 80-120 kg. In sows the nutrient requirement of copper is 5 mg/kg, both for lactating sows as sows in gestation[123]. Kirchgessner *et al*. have estimated the copper requirement of pregnant sows to be 6 ppm [123].

There is one recent multitrial analysis in which it was concluded that the optimum copper concentration in the feed of nursery pigs is between 50 and 250 mg/kg feed. More specifically it is 174 mg/kg when the average daily gain is taken as parameter and 119 mg/kg when looked at the average daily feed intake [293].

Pigs require copper for the synthesis of haemoglobin and synthesis and activation of oxidative enzymes. A deficiency results in abnormal hemopoiesis (microcytic hypochromic anaemia), poor keratinization and synthesis of collagen, elastin and myelin. This may result in bowing of the legs, spontaneous fractures, cardiac and vascular disorders and depigmentation. Copper can also have a preventive effect on the occurrence of diarrhoea in pigs [294, 295]. It also decreases the number of intestinal *E.coli* and *Streptococcus* spp [296, 297]. An EFSA literature search on the effects of copper on the microbiota reveals that the microbiota is already affected with low (<50 mg/kg feed) concentrations of copper. In this concentration mainly clostridia and coliform bacteria are altered. Higher concentrations (>170 mg/kg feed) also influences lactobacilli concentrations in piglets and growing pigs. The streptococci amount in the colon and faeces and the ureolytic bacteria amount in the colon is also reduced in fattening pigs, but not in piglets [104].

Increasing the copper concentration also resulted in a tendency towards less tail biting [294] and an improvement in zinc [297] and iron [298] digestibility.

Kirchgessner *et al*. found that sows fed 2 ppm of copper had reduced ceruloplasmin and farrowed more stillborn piglets than sows given 9.5 ppm [123].

When fed in pigs at 100-250 ppm copper has growth stimulating effects, probably because of its antimicrobial actions [123]. The optimum copper concentration for optimization of average daily gain and feed conversion ratio is 125-250 ppm in feed [299-301]. Copper sulphate is superior to copper sulphide for this manner [302].

2.1.2 Iron

The iron requirements decline with age: 100 mg/kg at a weight of 3-10kg, 80mg/kg for pigs 10-20 kg in bodyweight, 60 mg/kg at the weight of 20-50 kg and respectively 50 and 40 mg/kg for pigs with a weight of 50-80 and 80-120 kg. For pregnant and lactating sows the requirements are respectively 80 mg/kg feed [123].

Iron is needed as component of haemoglobin and in myoglobin, transferrin, uteroferrin, lactoferrin, ferritin and hemosiderin. Besides, it is important in several metabolic enzymes. In suckling piglets iron is needed to compensate for the low concentration at birth and in the sow's milk. The requirement is 7-16 mg/day or 21 mg/kg bw. This requirement is often met with an intramuscular injection with 100-200 mg iron, but oral administration within the first few hours, before closure of the gut to large molecules, of life will also meet the iron needs of the suckling pig [123].

2.1.3 Magnesium

The magnesium requirement is 0.04%in the feed for piglets, fattening pigs pregnant and lactating sows. The requirement for pregnant and lactating sows is respectively 0.7 and 2.1 gram per day[123].

In an old study Miller *et al*. determined that the minimal requirement for magnesium in very young piglets is 325 mg/kg feed. Piglets of this age however drink milk from the sow and supplementation should not be needed [303].

Magnesium is a cofactor in many enzyme systems and is constituent of the bone. A deficiency in magnesium is characterized by hyperirritability, muscular twitching, reluctance to stand, weak pasterns, loss of equilibrium and tetany which may result in death [123].

2.1.4 Manganese

The manganese requirements are 4 mg/kg, 3 mg/kg and 2 mg for piglets with a bodyweight of respectively 3-10 kg, 10-20 kg and 20-120 kg. For pregnant and lactating sows the requirement is 20 mg/kg feed [123].

Manganese is a component of several enzymes which are important in the metabolism of carbohydrates, lipids and is important for the synthesis of chondroitin sulphate which is a component in the bone matrix. Long term feeding of a deficient diet (0.5 ppm) results in abnormal skeletal growth, increased fat deposition, irregular or absent oestrus cycles, resorbed foetuses, small and weak piglets and a reduction in milk production [123].

The requirement of manganese in pigs has not been recently studied. In an old study by Grummer *et al*. there was a positive effect of increasing the feed concentration of magnesium from 12 ppm to 55 ppm [304]. In another old study it was shown that 50 ppm (versus 0 ppm) resulted in a growth retardation [305]. Besides the age of these studies, also very few different concentrations are investigated making it difficult to interpret the results.

2.1.5 Zinc

The zinc requirements are 100 mg/kg, 80 mg/kg, 60 mg/kg and 20 mg/kg for piglets with weights of respectively 3-10 kg, 10-20 kg, 20-50 kg and 50-120 kg. In sows 50 mg/kg zinc is advised [123].

Zinc is part of many enzymes including DNA and RNA synthetase and transferase, digestive enzymes and the production of insulin. It is therefore important for the metabolism of proteins, carbohydrates and lipids. The zinc requirement of breeding animals is not well established and might be higher due to foetal growth, milk synthesis, and tissue repair during uterine involution and sperm production in boars. Signs of a zinc deficiency in growing pigs include hyperkeratinisation of the skin and a reduction in growth. In gilts a zinc deficiency can result in a decrease in the number of pigs and the piglet bodyweight [123].

According to an old study by Hankins *et al*. the requirement is 26-31 ppm [306]. Hill *et al*. however did some very recent research about the zinc requirement of newly weaned piglets (18 days of age) and found that newly weaned piglets with the current growth rates need 75 mg/kg of feed [307].

The WUR has concluded that the growth of piglets increases when the zinc concentration of the feed increases from 33 to 48 ppm. Feed with a zinc concentration of 73 ppm resulted in the highest zinc concentrations in blood plasma. Overall, a concentration of 80 ppm would be sufficient for piglets [308].

2.1.6 Phosphorus

The requirement of available phosphorus declines with age in growing pigs: 0.55% for pigs with a bodyweight of 3-5 kg, 0.4% at 5-10 kg, 0.32% for a weight of 10-20 kg, 0.23 mg/kg for a weight of 20-50 kg and 0.19 and 0.15 mg/kg for weights of 50-80 and 80-120 kg respectively. In gestating and lactating sows the advised concentration is 0.35% [123].

Together with calcium, phosphorus is important for the development and maintenance of the skeletal system. The concentration of these minerals for a maximum growth rate is higher than the requirements for maximum production parameters [123].

Weller *et al*. evaluated three levels of phosphorus in growing pigs exposed to heat stress and concluded that sufficient levels of phosphorus seem to play a role in the oxidative phosphorylation and are thus important for animal performance during heat stress [309].

3. Cattle

3.1 Vitamins

3.1.1 Beta-carotene

Beta-carotene is a pro-vitamin of vitamin A. The conversion of beta-carotene to vitamin A is between 8:1 and 12:1 on weight basis. There is no requirement for dietary beta-carotene to replace vitamin A [310]. Of all carotenoids, beta-carotene has the most pro-vitamin A activity [125].

In ruminants beta-carotene is the main source of vitamin A. Beta-carotene is absorbed from forage and converted into vitamin A in the intestinal mucosa [311]. Besides the positive effects on disease and reproductive performance, supplementation of beta-carotene may also increase milk production [312, 313]. Ondarza et al found no increase in milk production, but they did find an increase in milk, fat corrected milk and milk fat yield in early-lactation and mature cows [314]. Rakes found no effect on both milk production and milk fat percentages [315].

Beta-carotenoids are taken up by neutrophils and lymphocytes of calves and act as antioxidants in the subcellular organelles to protect against oxidative damage [125]. The lymphocyte proliferative response against mitogens and the phagocyte and killing ability of bovine neutrophils can be enhanced by beta-carotene [125, 312]. Also in another study the stimulation of lymphocyte function, together with increased cytokine production and stimulation of the phagocytic effect of neutrophils and macrophage, has been shown.

The immunomodulating effects of beta-carotenoids are probably independent of their pro-vitamin A activity and might result in improved health, including mammary and reproductive health [312, 316]. Effects seen are a decrease in somatic cell count and number of (new) mammary infections [312, 317]. There are however also authors who found no effect on udder health [312].

In calves it has been shown that the immunomodulating effects of beta-carotene include an increased IgG concentration. This can result in fewer cases of clinical diarrhoea and upper respiratory diseases, together with an increase in growth rate [318].

Beta-carotene supplementation improves the phagocytic activity and intracellular killing capacity of blood neutrophils [319]. It also influences the immune system of the udder; in vitro tests with blood and milk phagocytic cells obtained from peri-parturient cows show that beta-carotene supplementation results in enhanced intracellular killing of *S. aureus* in these cells [320]. In another in vitro study from the same group it was concluded that beta-carotene may afford the mammary gland protection against infection through enhanced lymphocyte function [321]. Chew et al have shown a correlation between results from the California Mastitis Test(CMT) and the serum beta-carotene level; cows with less beta-carotene scored higher in the CMT [322]. Also the somatic cell count can be decreased in animals supplemented with beta-carotene [315].
In contrary to the authors above, Johnston et al proved in 1984 that higher beta-carotene levels were associated with an increased mastitis incidence [323]. Jukola et al and Oldham et al found no correlation at all between plasma beta-carotene concentrations and udder infections or somatic cell count [324, 325].

The antioxidant activity of beta-carotene depends on protection against oxygen medicated lipid peroxidation. It has a complementary effect on antioxidants as alfa-tocopherol, which inhibits free radical mediated lipid peroxidation, but has no effect against oxygen mediated peroxidation [131].

Beta-carotene seems to play an important role in the functioning of the reproductive organs. A lot of research has been done some decades ago about the role of beta-carotene apart from its role as a vitamin A precursor. In their review they describe that there have been several studies with positive effects or no clear effects. There was one study which showed negative effects. It is thus difficult to make a clear conclusion [312].

The effect depends, amongst others, on age, form of vitamin A in the diet and the concentration of beta-carotene. Positive effects that have been found include increased oestrus intensity, increased conception rates, decreased amount of services per concentration, decreased open days, a decreased incidence of cystic ovaries, decreased early embryonic death and abortion rates. Beta-carotene supplementation can decrease the amount of services needed per conception and decrease the interval between parturition and the first oestrus [312] and conception [315]. Aréchiga showed that pregnancy rates could be increased during summer, but not during the colder season [313]. Bindas et al found that beta-carotene supplementation did not affect the days to first breeding or services per conception. The days open were however reduced from 186 to 116 [326].

Beta-carotene accumulates in the corpus luteum (CL) and might affect the production of ovarian steroids [327]. Schweigert and Zucker have proposed that beta-carotene can be locally converted in to vitamin A; an increased vitamin A concentration in the follicles was related to better follicle development [328]. Injections with beta-carotene increase luteal size and luteal blood flow [329].

Beta carotene plays a part in the production of oestradiol in follicles and progesterone in the corpora lutea [330]. Also Graves Hoagland and all showed a correlation between beta-carotene status and bovine luteal function in vitro [331]. In contrary to Aréchiga, they showed that effects are found particularly during winter months, when plasma beta-carotene levels are low [331]. Besides it is important for the maturation and functional integrity of the oviduct, uterus and placenta [330] and can possible also have a role as antioxidant [332]. The embryo is said to be very sensitive to oxidative stress in the early stages [332].

Yildiz et al and Ataman et al showed a positive correlation between the plasma beta-carotene concentration and pregnancy [332, 333]. For optimum fertility in cows the plasma beta-carotene level should be 150-300 mg/dl. A deficiency can result in decreased progesterone output, delayed ovulation low oestrus intensity, high ovarian cysts incidence, embryonic mortality and abortions [330]. The increased abortion rate in cows with a beta-carotene deficiency is referred to as nutritional abortions, which can come together with an increase in cases of retained placenta [334]. Also other authors showed that beta-carotene supplementation can decrease the incidence of retained placenta and metritis [319].

In donor cows used for embryo collection, there was a positive correlation between the plasma beta carotene concentration and the embryo collection results [335].

It has also recently been shown that the serum beta-carotene level in premature calves with respiratory distress syndrome is too low; a lack of beta-carotene was thus considered to increase the incidence of premature birth of calves [336].

Also in goats it has been shown that beta-carotene improves ovarian activity, measured as total follicles, corpus luteum number and total ovarian activity [337].

In contrary to all the above mentioned studies in which a beneficial effect of beta-carotene on reproductive performance was found, there is also a study which shows that high levels of beta-carotene (500 – 700 mg/kg feed) can have adverse effects on fertility of dairy cows [338]. Wang et al,

Bindas et al, Jukola et al, Kaweluman et al and Akaordor et al found no beneficial effect of beta-carotene on reproduction [317, 324, 339-341]. Marcek et al concluded that supplementing beta-carotene did not influence the incidence of ovarian cysts, provided that the beta-carotene in the diet was sufficient [342].

3.1.2 Betaine

Betaine is not an essential vitamin and there is therefore no requirement mentioned in the Nutrient Requirements.

Overall milk production can be improved with the supplementation of betaine at a level of 100 gram per day. The supplementation however also decreased the percentage of milk protein and slightly altered the milk fatty acid profile. More research is needed to study the effects [343]. In another study no beneficial effect of betaine on the milk production was found [344].

A study in goats has shown that the supplementation of 4 gram betaine per kg concentrate resulted in an increase in milk production (fat corrected milk) during treatment and post-treatment. Other changes were an increase in milk fat and a decrease in roughage dry matter intake. The authors suggest that betaine results in an elevation in plasma acetate level resulting in more acetate for peripheral tissues [345].

The passage of vitamins through the rumen is always a complicating factor in the oral supplementation. There is one recent study which investigated the rumen passage of betaine. The authors suggest that betaine supplied to dairy cows dissolves in the rumen fluid and that more than 80% of it reaches the duodenum within 12 hours [346].

3.1.3 Biotin

There is no recommendation for adult cattle published by the NRC because of a lack of information. They do however advise 0.07 mg/kg DM for milk replacers in calves [347]. Biotin is synthesized in the rumen of cattle, depending on the availability of energy, but Zinn *et al.* advice to obtain 0.13 mg/kg bw of biotin in the feed [348].

Biotin positively affects the claws of dairy cattle [347, 349-354] and heifers [355, 356]. Several studies indicate that supplementing 20 mg/day improves hoof quality, but it is recommended to supplement cows over long periods (>6 months) to achieve an effective reduction in the risk of the occurrence of problems [312]. Also Potzsch *et al* concluded that a supplementation of at least six months is desirable, even though a positive effect on lameness scores could be achieved with shorter periods. In this study it is concluded that white line disease is more prevalent in multiparous cows and especially for cows in parities higher than five. The supplementation of biotin was very effective in multiparous cows, where the incidence of white line disease was decreased with 45%, but ineffective in primiparous cows [357]. The long term (until 14 months) supplementation of biotin at an amount of 20 mg per day results in a decreased incidence (-50%) of sole haemorrhages. There is however no effect on double soles, hoof wall grooves and heel horn erosions [358].
Also in beef cattle the use of biotin to prevent foot problems (10 mg/day) or to reduce laminitis has been proven efficient [312].

Biotin acts as a cofactor for various enzymes directly related to the synthesis of milk in the mammary gland [312]. Supplementing dairy cattle with biotin can increase the dry matter intake and the milk production [359] or the milk production alone [352]. Also Bersten *et al* found an increase in milk production; in a field trial where 20 mg of biotin was supplemented per day for 14 months resulted in a total increase of milk production by 487 kg and 25 kg of fat [358]. Also Majee *et al* found an increase

in dry matter intake (0.7 kg/day) and milk production (1.7 kg/d) when cows were supplemented with biotin per os at an amount of 20 mg/day. Supplementing a double concentration had no beneficial effect [360]. There are however also studies in which 10 or 20 mg/day could not influence these parameters [361]. Zimmerly & Weis found that the milk production was increased, but they did not report an increase in dry matter intake [362]. Milk production was unaffected when studied by Fitzgerald *et al*. [349]. The production of milk and its main components is not always improved by supplementary biotin because the response may be affected by various factors such as the composition of the diet, the stat of lactation and/or the status of biotin in the cow [363]. In a review published in 2006 eight studies were evaluated and in five out of these studies supplementary biotin increased the milk yield [364].

An effect of biotin (20 mg/day) on fertility was found in first lactation heifers, in which supplementation resulted in a significant lower number of days from calving to conception and a significant decrease in the number of inseminations needed [358].

Biotin also appears to affect the metabolism of dairy cows post-partum. Supplementation with biotin before (20 mg/day) and after (30 mg/day) parturition results in an increase in blood glucose concentration and a decrease in lipid metabolism [365]. In a review from 2006 it can be found that biotin supplements increased plasma glucose in two out of four studies [364]. More research is needed in this field. Supplementing biotin in high-producing dairy cattle is also suggested as an aid to reduce the occurrence of fatty liver syndrome [312].

3.1.4 Choline

Recommendations from the NRC are only known for calves: 260 mg/liter milk or 1000 mg/kg DM [347]. In a very old study the requirement of calves was determined at 1.733 mg/kg of DM milk replacer. Deficiencies could be induced in the animals used in this study [366].
There is little information for the requirement of adult cattle. Pinotti *et al*. concluded that, although the data are insufficient to establish real requirements of choline in dairy cattle, supplementation of between 12 and 20 mg/day would be appropriate for dairy cattle in transition [367].

It is known that large amounts of choline are degraded in the rumen [312]. Therefore, in most studies discussed below rumen-protected choline is used.

In calves signs of deficiencies include muscular weakness, fatty infiltration in the liver and renal haemorrhage [311].

Zom *et al*. showed that supplementing 14.4 gram of choline per day for the period of three weeks before parturition until six weeks after parturition increased the intake of dry matter and decreased the concentration of triacylglycerol. There was no effect on milk production, milk fat or lactose [368]. There are two studies in which the milk production was increased, but there was no effect on milk fat [369, 370]. An increase in milk production was also found specifically during the first month after calving (20 mg/day), but without an effect on milk fat, plasma glucose or the concentration of beta-hydroxybutyrate or fatty acids in plasma [371]. In an old study it was concluded that there was no effect of choline on the milk production or milk fat percentage [372]. In a meta-analysis of different studies it is concluded that choline can affect the dry matter intake and milk production, but that there is no consistent effect on milk fat percentage [373]. Weiss summarized the results of 12 experimental studies in milk production when rumen protected choline was given during the first two months of lactation. The additional supply of choline is then considered profitable [374].

Cooke *et al.* found that supplementation with choline (15 g/day) resulted in a decrease in the concentration of fatty acids in plasma and a decrease of TAG disposition in the liver in cows that were

restricted in feed [375]. Goselink *et al*. suggested that the reduction in liver triacylglycerol by improved fatty acid processing and very low density lipoprotein synthesis and benefit on hepatic carbohydrate metabolism [376]. It is hypothesized that choline aids the transport of mobilized free fatty acids from adipose tissue through the liver to the mammary gland [377].

In a recent study from Lima *et al*. conflicting results are found when 15 gram of choline was supplemented per day. In cows this vitamin decreased the prevalence of ketosis and mastitis, as well as the severity of the mastitis occurring. In heifers however, supplementing choline resulted in both negative and positive effects on health[378].

In beef cattle supplementing choline has a positive influence on growth, without any negative effects on the quality of the carcass [379]. In calves an increase of choline from 0 to 0.25% in the feed results in an increased daily growth of 11.6%. The daily feed intake was not altered, but feed conversion improved with 6.8% [380].

3.1.5 Folic acid

There is no recommendation about the folic acid requirement published by the NRC for either calves or adult cattle. In dairy cattle folate is synthesized in the rumen and folic acid administered orally is often degraded in the rumen. Protected folic acid would thus be advised [311]. The ruminal production is likely to be sufficiently high in order to avoid a deficiency but probably not high enough to guarantee optimum production [311, 381]. Unfortunately, there is insufficient evidence to determine requirements and make recommendations for the use of folic acid in practical conditions [311].

In weaned calves it is shown that intramuscular injection of 40 mg of folic acid can contribute to an increase in growth and increased concentration of haemoglobin [382]. Folic acid (1 mg/kg/day) can also contribute to the repair of skin diseases such as alopecia in calves. This is however only shown in calves which were deficient of this vitamin [383].

In dairy cows supplementing folic acid around parturition (2.6 gram/day) results in an increase in milk production (+3.4 kg/day) and milk crude protein yield (+0.08 kg/day) [384]. In another study folic acid is not only supplemented in the transition period, but during the entire lactation (2-4 mg/kg/day). An increase in milk production could in this study only be observed in cows in the second lactation or higher. In heifers a slight reduction in milk production was found in the first hundred days of lactation [385]. In another study there was no effect of three or six milligram folic acid per kg bodyweight per day on the milk production in dairy cows. The concentration of crude protein and casein in the milk however increased, while the lactose concentration in milk decreased [386].

Supplementing folic acid during pregnancy did not influence birth weight, feed intake or growth of the calves during the first ten weeks of life. There was however in an increase in milk production in the cows [381].

3.1.6 Niacinamide

The NRC guidelines do not mention niacinamide as a vitamin, but they do mention niacin. Niacinamide is an amide of niacin and both play a part in the same functions. Niacin is produced in the rumen of cows, but there are also some studies done in which the supplementation of niacin has been investigated. When niacin is administered orally, the concentration in the small intestine increases which indicates that at least part of the administered amount passes the rumen. For calves with an inactive rumen niacin is an essential component of the milk replacer. The recommendation for calves is 10 mg/kg DM niacin in milk replacer [347]. For adult cows no recommendations are mentioned. Flachowsky concludes that there is a lot of variation between the outcomes of different trails. This

could be explained by differences in feed, milk production, moment of lactation, age, body condition score and the amount of niacin that is administered [387].

In calves it was shown that a deficiency of niacin resulted in diarrhea within 48 hours. There was immediate improvement the day after administering niacin 6 mg oral or 10 mg intramuscularly [388].

The effect of niacinamide supplementation on the milk production in cows varies; two investigators showed that niacinamide supplementation results in an increase in milk production and total milk protein [389, 390], whereas in another study no significant effect was found on the dry matter intake, milk production, milk composition and plasma concentrations of NEFA and BHBA [391]. In the study by Cervantes *et al*. this was accompanied by a decrease milk fat [389]. Jaster & Ward also found a decrease in BHBA in serum, a higher blood glucose concentration and a lower concentration of free fatty acids in plasma when niacinamide was supplemented for a sufficiently long period (12 weeks) [390]. Minor *et al* found that supplementing cows with 12 g niacin per day does not result in an increase in milk production or blood parameters. There is however an increase in pre-partum intake of dry matter and energy and an improvement in energy balance [392]. In another study it was found that supplementing 12 gram nicotinic acid per day increased milk yield, solid-corrected milk and 3.5% fat-corrected milk. This was accompanied with a decrease in crude protein and true protein in milk [393].

Studies in which niacin is used also show varying results; Riddel *et al*. showed a positive influence of niacin on the milk production and milk fat percentage, especially in fresh cows and cows fed a diet with a natural protein source (versus urea) [394]. Also Lohölter *et al*. showed an increase in milk production and an increase in total milk protein and lactose concentrations. The percentage of fat and protein per liter milk decreased [395]. In another recent study there was also a significant increase (4.44%) in milk production, a significant decrease (2.48 vs. 3.93) and a trend for a lower NEFA concentration [396]. Dufva *et al*. found a positive influence on the milk production in combination with an increase in the blood glucose concentration and a decrease in the concentration of NEFAs and BHBA. The composition of the milk was not significantly altered [397]. In a review it is concluded that niacin supplementation leads to an increase in milk production (3-4%), but that there is insufficient evidence to show a change in milk composition [398]. A decrease in BHBA concentration and an increase in blood glucose concentration were found by several authors [399, 400]. Ghorbani *et al*. found there was no effect on the milk production [399]. At last, there is one study in which no effect on the milk production, milk composition, body weight, body condition score (BCS) or energy balance was found. A very high dosage results in a decrease of the NEFA concentration post-partum, but could also result in a decreased dry matter intake [401].

The above effects of niacin have been summarized in the chapter of Casals & Calsamiglia as follows: niacin stimulates microbial protein synthesis, the production of propionic acid and digestion of cellulose. On the metabolic level, niacin participates in lipid and energy metabolism. Supplementation with niacin leads to an increase in the concentration of glucose in blood, a decrease in the concentration of beta-hydroxybutyric acid and free fatty acids in plasma, which are indicators of its activity as gluconeogenesis stimulator [311]. Calsamiglia & Rodriguez added for dairy cows: niacin participates in lipid and energy metabolism, reducing the risk of ketosis and fatty liver, and occasionally improving the production level and composition of milk [312].

In one study it was shown that the effect of niacin on the milk production depends on the body conditions score (BCS); a positive effect was found in heifers and cows with a BCS of three or four, but production decreased after niacin supplementation in cows with a BCS of two [402].

In bulls niacin is important for growth, especially through its effect on the propionate concentration in the rumen, the feed intake and the feed efficacy [387]. In another study published by the same author it was concluded that the effect occurs particularly in bulls weighing less than 300 kg, bulls fed a diet with low protein or during periods with feed changes [398].

Niacin supposedly also affects the microbes in the rumen. The microbial synthesis increases after niacin supplementation when a natural source of protein is fed, but not when the main protein source is urea [394]. Aschemann *et al.* examined the effect of niacin when cows were fed a diet with a negative nitrogen balance. It was found that additional administration of niacin resulted in higher blood glucose concentrations and a better utilization of nitrogen [403]. These results have been repeated in a second study conducted by the same study group and it was concluded that the increase in nitrogen utilization can be explained by a change in the composition of the micro flora in the rumen [404]. Also in other studies it has been shown that niacin supplementation results in larger amounts of bacteria in the rumen and a better digestibility of feed [387, 405].

Niacin also seems to be important for the fertility of cows. Flachowsky found that the number of open days decreased in cows supplemented with this vitamin [398].

A review from 2009 describes that niacin in synthesised by microbes in the rumen but that the amount that is produced varies depending on the diet. Degradation might occur before niacin reaches the intestines. Supplementation of niacin would be particularly interesting during periods of metabolic stress, such as during ketosis [406]. This is confirmed in sheep by Khan *et al*., who demonstrated that administration of niacin during a period of at least four weeks results in a transition of type I to type II muscle fibres. These muscle fibres are able to utilize fatty acids [407]. In an earlier study it was however suggested that niacin results in an increase in the propionate concentration and a decrease in the butyrate concentration [408].

In a recent study Rungruang *et al*. investigated the use of niacin to improve the thermo tolerance of winter-acclimated lactating dairy cows exposed to moderate thermal stress [409]. In another study it was concluded that administration of extra niacin during periods of heat stress did not result in an increase of the milk production or the intake of dry matter. The cows did however have a lower rectal and vaginal temperature than control animals [410].

3.1.7 Vitamin A/ Retinol

The amounts recommended by the NRC for beef cattle vary for cows in gestation, lactating cows, feedlot calves and beef cattle and are respectively 2.800, 3.900, 2.200 and 3.900 IU/kg dry matter. The recommendations for suckling calves are 9.000 IU/kg DM milk replacer and 4.000 IU/kg DM starter feed [411]. For dairy cattle the recommendations are 110 IU/day for lactating and dry cows [347]. The optimum amount of vitamin A in the feed of cows lactating or suckling the calf is however higher: 10.000 IE/kg DM (200 IU/kg bw) [311]. The recommendations for cows in the dry period are preferably also increased with 50% compared to the NRC guideline [412]. Weiss et al. concluded the same after evaluating several studies in which the effects of supplemental vitamin A on milk production, mammary gland health and immune function were evaluated [413]. In feedlot calves, the concentration might be increased to 4.000-6.000 IU/kg DM during stressful periods such as weaning or arrival at the feedlot after a long journey [411].

The vitamin A requirement of growing calves is estimated at 25.000 IE per 100 kg of bodyweight[414]. For beef cattle the requirement is not easily determined because it is depended on the bodyweight and the composition of the diet. Research shows that relatively low concentrations are needed of approximately 10.000 IE per 100 kg bodyweight [415].

The vitamin A status of calves depends greatly on the intake of vitamin A through colostrum. If the vitamin A levels remain low after parturition, the calf's health and its subsequent growth may be negatively affected. Cows fed ration rich in vitamin A during the dry period produce more colostrum with a greater vitamin A concentration [312].

Vitamin A is one of the important vitamins for beef cattle because it is essential for normal growth and development and development of tissue and bones. Additionally it also plays a role in vision, embryo development, fertility, immunity, maintenance of homeostasis and a wide range of mammary gland cell functions. Deficiencies usually occur when a high concentration of concentrates is fed, when the feed has been exposed to sunlight or high temperature or after a long storage period for the feed [311]. Vitamin A deficiency has been associated with loss of vision, reduced growth and development, changes in spermatogenesis and in the maintenance of skeletal and epithelial tissue and a reduction in reproductive and immune functions [416]. It has been reported that a vitamin A deficiency in calves can result in orthokeratotic dermatopathy [417]. The negative effect of a vitamin A deficiency on the mucosa has been linked to a persistent diarrhea with secondary *E.coli* infections in beef calves. At pathology the intestines were characterized by intestinal capillary congestion, cell necrosis and exfoliation of epithelial cells in the mucosa [418]. A vitamin A deficiency in gestating cows leads to an increase in the incidence of abortions, retained placentas, and increased calf morbidity and mortality rates, as well as a reduction in fertility [327]. It has already been shown that supplemental vitamin A resulted in a decreased incidence of retained placentas and milk fever (plus 200 IU/kg bw) and a decrease in somatic cell count postpartum (1660 IU/kg bw). Supplementing vitamin A during the dry period would result in a reduced incidence of mastitis in the dry and reproductive periods [312]. Also O'Rourke linked lower plasma concentrations of vitamin A (<80 µg/ml) with an increased severity of clinical mastitis [419]. In 2007 Also Kankofer found that vitamin A and its pro-vitamin beta-carotene, can influence the process of foetal membrane retention [420].

Increasing the vitamin A administration during the dry period would result in a better immunity, mammary health and postpartum reproduction problems [311]. Stimulation of the immune system reduces mammary and reproductive problems, both in the dry period as during lactation [312]. Oldham *et al*. however did not find an effect of increased vitamin A supplementation around the time of the dry period on the prevalence of mastitis. The cows receiving 170.000 IU vitamin A per day did however have a higher fact corrected milk production than cows receiving vitamin A at an amount of 50.000 IU/day [325]. An old study of Meacham *et al.* determined that the administration of vitamin A (16.000 or 40.000 IE /day) resulted in better survival rates of calves and better fertility of dairy cows [421]. Literature suggests that production can also be boosted with the use of vitamin A: an increase in milk production (plus 250 IU/kg bw) and increased heat detection (1660 IU/kg bw). Using enormous amounts of vitamin A (1.000.000 vs. 100.000 IU/day) did not result in an increased progesterone concentration of improvement of production parameters, but this could be due to the already high concentration of vitamin A in the control group [422].

Vitamin A is also important for the immune system. It plays an important role in the passive immunity of dairy cattle since it intervenes directly in defence mechanisms through the maintenance of functional epithelial tissue and stimulation of the immune function [312]. A low vitamin A diet compromised the serum IgG1 responses against inactivated BCoV vaccine, which suggested suppressed T-helper 2-associated antibody (IgG1) responses. Thus, low vitamin A diets may compromise the effectiveness of viral vaccines and render calves more susceptible to infectious disease [423].

Despite the importance of vitamin A, increasing the concentration of vitamin A in the feed should be done cautiously because an excessive amount of vitamin A can lead to a reduction in the quality of the meat in beef cattle [424].

3.1.8 Vitamin B_1 / thiamine

The NRC does not have recommendations for vitamin B_1 in adult cattle because the amount produced in the rumen is probably enough to meet the requirement of 28-72 mg. A deficiency will only occur when rumen function is impaired, e.g. when the ration contains high levels of concentrate or is rich in quickly fermentable carbohydrates [311]. For calves a concentration of 6.5 mg/kg DM of milk replacer is advised [411].

Thiamine is a coenzyme which participates in many metabolic functions [311]. A vitamin B_1 deficiency can result in polio encephalomalacia.

There is one study in which supplementing 150 mg vitamin B_1 per day in dairy cows has been studied; there is a trend towards a higher milk production and better milk composition. This effect is particularly seen when the feed contains low levels of fibres or high concentrations of non-fibre carbohydrates [425].

3.1.9 Vitamin B_2 / riboflavin

For vitamin B_2 there is no recommended level in feed for adult cattle, because this vitamin is synthesised in the rumen. Deficiencies have only been seen in pre-ruminant animals. For calves the recommended concentration is 6.5 mg/kg DM in milk replacer [411].

There is only limited information available from the literature. In a very old article Draper *et al.* describe that calves require vitamin B_2 in the first ten weeks of life. The advised concentration is 1 µg/kg DM [426].

In calves and dairy cows vitamin B_2 supplementation results in an increase in the number of neutrophils and an increased neutrophil activity. In calves this effect was seen after the administration of 10 mg/kg bw and in cows when at least 5 mg/kg bw was administered [427].

Sato *et al* also found increased neutrophil activity after a single intravenous injection with vitamin B_2 (2.5 or 5 mg/kg bw) or a three day administration with 2.5 mg/kg bw. In the same study the injection of 2.5 mg/kg bw vitamin B_2 at three consecutive days resulted in a decrease in somatic cell count until 14 days after the initial injection. There was however no bacteriological cure [428].

3.1.10 Vitamin B_5 / D-panthenol

D-panthenol is not mentioned in the NRC guidelines, but pantothenic acid is. Panthenol is the alcohol compound of pantothenic acid. There is no recommendation for adult cattle because this vitamin is produced in the rumen. In calves the recommended concentration is 130 mg/kg DM of milk replacer [411].

In a recent study it has been demonstrated that supplementing pantothenic acid to adult cows is only beneficial when a compound is used that is rumen protected [429], although another study from the same group concluded that despite the fact that 80% of the administered pantothenic acid does not reach the duodenum, oral supplementation does result in an increase in the plasma concentration [430].

Sheppard & Johnson have investigated some aspects of pantothenic acid in calves and they suggest that a deficiency can be provoked, but more research would be needed to acquire a recommended dosage [431].

3.1.11 Vitamin B_6 / pyridoxine

The NRC has no recommendation for adult cows because this vitamin is produced in the rumen. In calves, the milk replacer should contain 6.5 mg/kg DM [411].

Pyridoxine or vitamin B_6 is coenzyme which actively participates in the metabolism of amino acids, and requirements are therefore established according to the quantity of protein intake [137].

In the literature only one study can be found Johson *et al*. showed that calves can develop deficiency signs when not given vitamin B_6. Administering first 10 mg/day subcutaneously and later 100 mg/day orally was a good therapy [432].

3.1.12 Vitamin B_{12} / cyanocobalamin

For cattle there is no NRC recommendation for vitamin B_{12} because it is produced in the rumen, providing cobalt is available. Orally administered vitamin B_{12} is for 90% degraded in the rumen. It is shown that the oral administration of cobalt is effective in rising the vitamin B_{12} concentration in the liver [433]. For cobalt the NRC recommends 0.10 ppm in feed, but more recent data indicate that this value should be raised [311]. In a study by Stemme *et al.* it was concluded that a concentration of 0.13 mg cobalt per kg DM is sufficient for dairy cattle [434]. For calves the NRC advices to use 0.07 mg/kg of DM in milk replacer [411].

In an article from 2001 contradicting results are described about the effect of intramuscular injections with vitamin B_{12} (20 mg). Some researchers found an increase in milk production, but others could not reproduce these results [435]. In a more recent study it was shown that oral cobalt supplementation (25 or 75 mg/day) or weekly vitamin B_{12} injections (10 mg per week) did result in better vitamin B_{12} status. The vitamin B_{12} concentration increased in both the milk and liver for both treatment and additionally in plasma for the animals given the vitamin B_{12} injections. There was however no influence on production parameters, which is possible caused by the fact that the control diet might also contain a sufficient concentration of cobalt (1 mg/kg dry matter) [436].

Croom *et al*. showed that even though vitamin B_{12} injections did not correct the decrease in milk fat synthesis associated with low fibre diets, an injection with 150 mg of vitamin B_{12} resulted in an elevated B_{12} plasma concentration which was 1200 times the pre-dose concentration four hours after administration and seven times the pre-dose concentration after one week [437].

The growth of calves [438] and heifers [439] could not be increased with supplements containing this vitamin.

The effect of administering cobalt in dairy cattle had varying results [440] or no effect at all on the milk production [436].

Cobalt is important for the maintenance of a normal immune system, which is proven for infections with *Ostertagia ostergia*. A deficiency results in more severe infections with this parasite [441].

Intravenous injections with cobalt (175 mg/day) or oral administration (3.5 gram/day) results in a decrease of the concentration in fatty acid desaturation products in both blood and milk [442].

3.1.13 Vitamin C / ascorbic acid

Vitamin C is endogenously synthesized in cattle and it is generally accepted that adult cattle synthesize sufficient ascorbic acid to cover their vitamin C requirement [311]. The NRC has no recommendations. The INRA advices to incorporate 100 mg/kg DM for veal calves [443]. The requirement of calves

depends on the genetic predisposition of the individual animal and on the environmental condition, such as housing. The recommended concentration for milk replacer is 2000 mg of ascorbic acid [444].

Oral administration of unprotected vitamin C in ruminating cows is not effective because this vitamin will be degraded in the rumen. When rumen protected vitamin C is used however, this results in increased plasma concentrations, which indicates there is absorption of this vitamin [445].

Even though vitamin C is produces endogenously, previous studies have showed that the concentration of vitamin C decreases during periods of stress and disease. The requirements are possibly not met during these periods [446]. As an example, Padilla *et al*. showed that the plasma vitamin C concentration decreased significantly during periods of heat stress and they concluded that dietary supplementation with vitamin C may be beneficial for lactating cows in hot weather [447]. Calves housed in a cold environment might also experience a decrease in plasma vitamin C concentrations [448].
Also experimentally induced mastitis resulted in a decrease in plasma and milk concentrations of vitamin C. Additionally, the milk vitamin C concentration of the affected quarter was correlated to the duration of mastitis, peak body temperature, number of colony-forming units of *E.coli* isolated from the udder and loss in milk yield. There was also a relation between these parameters and the plasma concentration of vitamin C, but this was statistically weaker [449]. The vitamin C requirement also increases during lactation because of the large production of lactose in the mammary gland [450]
In calves it was shown that housing in metal indoor pens resulted in a lower plasma vitamin C concentration compared to calves housed in hutches [451].
Besides, calves are not capable of producing vitamin C in the first weeks of live [452]. They depend on colostrum for the intake of vitamin C. The amount of vitamin C in colostrum depends on the preservation process and may be insufficient in some cases. Three studies showed that supplementing vitamin C (3000 mg/day in the first week, 2000 mg/day in the second week, 1000 mg/day until feed intake is 1 kg) to calves results in a reduced incidence of diarrhea and a decrease in mortality [452-454]. This can be explained by the role of vitamin C in the maintenance of normal mucous membranes, such as in the intestines [452].

Vitamin C is also important for the immune system. A deficiency of this vitamin results in a lower concentration of lymphocytes, monocytes and fibrinogen and a higher concentration of albumin [455]
Cummins & Brunner showed that supplementing calves that did not receive colostrum with vitamin C resulted in a higher concentration of IgG. In calves that were not deficient of vitamin C, supplementing 1.75 gram of vitamin C per day did not have an immunostimulatory effect[453]. In a slightly older study it was shown that supplementing vitamin C at a concentration of 10 mg/kg feed resulted in both positive and negative effects: reduced ocular and nasal discharge but impaired neutrophil function. The co-administration of vitamin E however resulted in compensation of these effects [456]. The intramuscular administration of vitamin C at the time of antibiotic treatment in calves with bovine respiratory disease may reduce the mortality rate [457].
In dairy cows the administration of two intravenous injections with 25 mg vitamin C, three and five hours after the experimental induction of acute mastitis resulted in an increased milk production recovery (9% higher) and tended to reduce the extend of rumen stasis [458]. The subcutaneous administration of vitamin C (25 mg/kg) for five consecutive days resulted in a faster recovery rate of clinical mastitis in cows that were also treated with an intramammary antibiotic [459].
The positive effects attributed to vitamin C on the immune response are explained by the stimulation of neutrophils. In some cases this also results in a reduction of mastitis indicators [312].

Vitamin C is also important for fertility; ascorbic acid therapy helped to maintain pregnancy in about 60% of the cows that had difficulty maintaining pregnancy in the past. There is however no effect on cows with cystic ovaries or other anatomical abnormalities. In bulls a vitamin C treatment was effective as treatment of sterility in animals that were heavily utilized and had a decline in ability to impregnate cows. Low grade semen was changed in character from a thin, watery, lifeless substance to normal appearing, viscous semen with highly motile sperm [327].

3.1.14 Vitamin D_3 / cholecalciferol

The NRC recommendation for adult dairy cattle is 30 IU/kg bw or 21.000 IU/day[347]. In beef cattle the recommendation is 275 IU/kg DM for gestating or lactating cows and grower or finisher calves. In young calves the advised concentration in milk replacer is 600 IE/kg DM[411].

In dairy cattle supplying animals with 70 IU/kg bw resulted in slight improvements of reproductive efficiency and milk production. The current NRC requirement (30 IU/kg bw) is probably adequate, but the low cost of supplemental vitamin D and the potential for increased milk production and reproductive efficiency support the field practice of feeding about 1.8 times the NRC requirement [413].

The pharmacokinetics of vitamin D_3 change with the age of the animals: cows starting the second lactation showed a significantly longer plasma half-life of 25-OHD3 than cows starting the third or higher lactation. The influence of the lactation number on the pharmacokinetics is, according to Wilkens *et al.*, directly related to age. This effect occurred irrespective of the dosage that was given, which was three, four or six milligram per day orally [460].

It is shown that cows fed organic feed need additional vitamin D_3 when not exposed to enough sunlight, especially during the winter. During summer and autumn cows that graze are exposed to sufficient amounts of UV light to produce enough vitamin D_3 endogenously [461].

Vitamin D is involved in the active transport of calcium and phosphorus across the intestinal epithelial cells and boosts the action of parathyroid hormone in reabsorbing bone calcium [312]. It is also believed to be important in maintaining calcium homeostasis around parturition, but the presence of sufficient levels of vitamin D or its precursors is not the only factor responsible for this process [462]. Horst and Littledike showed that the plasma concentrations of phosphorus and calcium increase after vitamin D has been administered by intramuscular injection. The concentration used was 15 million IU per injection and the injections were given at a weekly interval. The phosphorus concentration increased after the second injection, the calcium concentration after the fourth injection [463].
In one study it was found that the use of vitamin D_3 to prevent milk fever was effective in cows that were previously diagnosed with this disease but not in cows which were diagnosed with milk fever for the first time [464]. In another study it was shown that concentrations of 20-30 million IE of vitamin D per day for three to eight days pre-partum was effective in the prevention of about 80% of expected milk fever cases in cows with a history of milk fever. Also in this study there was no significant effect of vitamin D supplementation on the occurrence of milk fever in animals with no previous milk fever cases [465].

Cows fed alfalfa hay under confined conditions without vitamin D supplementation had a higher incidence of calves with clinical rickets and muscle weakness compared with cows receiving vitamin D supplementation. Vitamin D supplementation also influenced time of first postpartum oestrus and calving interval but not services per conception [327].

Vitamin D_3 was not effective in preventing the occurrence of paresis in calves when administered eight days before the expected farrowing date and there was no effect of the occurrence in retention secundinarum in cows [466].

In a review of several studies the conclusion was drawn that cows supplemented with 50-70 IU/kg bw produced more milk and generally consumed more dry matter than animals not supplemented or animals supplemented with very high concentrations (100-140 IU/kg bw) of vitamin D [467].

It has also been demonstrated that vitamin D_3 is involved in maintaining immune function by stimulating humoral immunity and by inhibiting cell-mediated immunity [312].

3.1.15 Vitamin E / alfa-tocopherol

The recommendation is 20 IU/kg DM (0.8mg/kg bw / 500 IU/day) for lactating cows and 80 IU/kg DM (1.6 IU/kg bw / 1000 IU/day) for dry cows[347]. Some authors suggest that pre-partum cows should receive higher concentrations of vitamin E (1000 IU/day) to increase the concentration of vitamin E in colostrum and minimize the incidence of postpartum metabolic disorders [311]. A review done in 1998 showed that the vitamin E requirement mentioned by the NCR is insufficient; the minimum requirement should be 25-75 IU per kg dry matter (DM), but most data suggest that diets that include 75 to 190 IU vitamin E per kg DM for dry cows and 25 to 50 IU of vitamin E per kg DM for lactating cows are beneficial [413]. The intake of vitamin E varies greatly depending on the feed; it is estimated to be 2500 IU for calves at the pasture, 1500 IU per day for cows fed silage based diet and 400 IU for cows fed hay based diet. Also the storage of feed is important: a long period of storage before feeding has a negative influence on the vitamin E concentration of the feed [413]. Vitamin E deficiencies are frequently observed in peri-partum dairy cows [419].

A study done in Belgium showed that 18% of all dry and early lactating organic cows were at risk of a vitamin E deficiency, where a plasma concentration of three microgram per millilitre was set as minimal level to avoid health risks [468]. Also in another study it was concluded that organic cows cannot fulfil their vitamin E (and vitamin A) requirements at the time around calving [469].

The NRC recommendation for young calves is 15-60 IU/kg DM. For weaner calves the specific recommendation is 50 IU/kg DM[411]. For new born calves it is very important to administered colostrum with a sufficient amount of vitamin E during the first 24 hours. Placental transfer of this vitamin is limited and intestinal absorption is reduced after 24 hours [312].
For feedlot calves at the age of 3-4 weeks a higher concentration of 400-500 IU/day is advised because the supplementation during this critical period can have positive effects on growth, conversion index and incidence of disease [411]. The use of additional vitamin E also seems of benefit when calves just arrived at the feedlot. This does not improve daily weight gain or the ingestion of feed, but it does decrease the incidence of respiratory disease [470].

Vitamin E is a powerful antioxidant agent and is integrated in cellular membranes. In this position it can protect these membranes against peroxides which are generated by phagocytosis in the course of an inflammatory process. In this way, vitamin E contributes to maintaining the integrity of cellular membranes [312].

In dairy cows the effect of vitamin E on udder health has been intensively studied. The incidence of clinical mastitis increased in over half the herds when the supply was below 23 IU/kg diet. In several studies positive effects on udder health have been proven when 1000 – 4000 IU/ day are administered: a decrease in incidence of clinical mastitis, a decrease in cell count and an improvement of the oxidative stability of the milk. The author of this review concludes that supplementing vitamin E during the last

part of the dry period is beneficial. The recommended dosages are 1000-3000 IU/day during the dry period and 500-1000 IU during lactation. At farms with a known history of udder health problems, the upper level of 3000 IU/day would be advised during the dry period [471, 472]. Also in another review it is concluded that supplementing vitamin E at a concentration of 1000 IU/day results in a decreased incidence of mastitis [473]. Also a review written in 1998 results in the same conclusion [413]. Smith *et al*. showed a reduction in the incidence of mastitis of 37% and a decrease in duration of 62% after supplementing 1000 IU/day during 60 days dry period [474]. O'Rourke referred to a book in which a trial was discussed in which a meta-analysis resulted in the conclusion that vitamin E supplementation on average resulted in a 14% reduction in the risk of intramammary infections, a reduction in milk somatic cell count by a factor 0.70 and a 30% decrease in the risk of occurrence of clinical mastitis [419]. Weiss *et al*. supplemented cows in the two weeks prior to parturition with 100, 1000 or 4000 IU/day and found that the highest concentration reduced the incidence of clinical mastitis by 80% and the incidence of intramammary infections by 60%. The effect of vitamin E on the prevalence of clinical mastitis was most prevalent in primiparous animals [475]. A decrease in somatic cell count was also shown for the supplementation of 2000 IU of vitamin E per day (compared to 1000 IU/day) during two weeks before and one week after the expected calving date [476]. The positive effects on udder health can possible be explained by the fact that the normal decrease in neutrophil chemotaxis around parturition is prevented by sufficient levels of vitamin E [477, 478]. In one study it is however shown that supplementing 3.000 IU/day is a risk factor for the development of clinical and subclinical mastitis [479]. According to this author this is possible caused by an increase in free radicals after administration of 3.000 IU vitamin E per day [480]. Consequently, Politis *et al*. studied the relation between α-tocopherol concentrations in plasma on the incidence of mastitis and biomarkers for oxidative stress. They concluded that blood α-tocopherol is inversely related to certain biomarkers of oxidative stress in the postpartum period and incidence of mastitis. The reduction in incidence of mastitis is however not mediated through a reduction in the levels of various biomarkers of oxidative stress [471].
At last there was a study in which the supplementation of a single intramuscular injection of 3000 mg vitamin E did not have any effect on the occurrence of clinical mastitis [481].

The administration of vitamin E also positively influences the fertility postpartum [482]. In a meta-analysis [483] and review [473] it is concluded that supplementing vitamin E during the dry period results in a decrease in the incidence of retention secundinarum. According to Leblac *et al.* administering vitamin E (subcutaneously) one week before the expected calving does result in a decreased incidence of retained placentas in cows with a marginal vitamin E status, but had no effect in cows with an adequate serum vitamin E concentration. There was no effect on metritis, endometritis or other diseases [484]. Campbell & Miller found that the supplementation of 1000 IU/day during 42 days (dry period) resulted in a decreased time to return to oestrus (from 60 to 42 days) and a reduction in the days to conception (from 71 to 62 days) [485]. Also Baldi *et al.* concluded that the supplementation of 2000 IU vitamin E per day during two weeks before and one week after calving resulted in a decreased number of services and days to conception [476]. Several other authors have published similar results [312]. In a review it was concluded that the incidence of retained foetal membranes can be decreased with the supplementation of 1000 IU of vitamin E per cow per day to dry cows, when the amount of selenium in the diet is adequate [413]. A single injection with 3000 mg vitamin E two weeks before the expected calving date resulted in a decreased incidence of retained placentas and metritis [481]. Horn *et al* however found that supplementing synthetic or natural vitamin E (1000 IU/day), starting six weeks pre-partum, does result in an increase in the α-tocopherol concentration in plasma, but has minimal effects on reproductive efficiency in beef cows [486]. LeBlanc *et al* showed the relation between vitamin E and retained placentas in a different way: when accounted for the effects of parity, season and

twins, an increase in α-tocopherol of 1 µg/ml in the last week pre-partum reduced the risk of retained placentas by 20%. The same authors found that a single injection of 3000 IU RRR-tocopheryl acetate results in an average increase in the α-tocopherol concentration with 0.4-0.5 µg/ml [487].

The effect of vitamin E on lung health in calves has been studied by several authors. Stanford *et al*. did not find any effect when using 550 IE per day. Higher concentrations were not used because he did not consider this economically viable [488]. When 1140 IE per day was used in another study there was a positive effect, but more research is needed according to this author [489]. Carter *et al*. showed that the use of 2000 IU per day decreases treatment costs related to lung diseases [490]. Duff and Gaylean first concluded that vitamin E at doses of greater than 400 IU per animal per day seemed beneficial for increasing average daily gain and decreasing bovine respiratory disease morbidity, but later conclude that the use of vitamin A has positive effects on morbidity, but is perhaps less effective as an aid to increase the daily weight gain in case of respiratory diseases [491].

Dietary or parenteral supplementation of vitamin E to dairy cows during the peri-partum period has consistently improved the function of neutrophils [413]. A general effect of vitamin E on the immune status of calves seems possible, but cannot be proved significantly by Cipriano *et al*. [492]. In another study there was a trend for a higher concentration of IgG after vitamin E supplementation. The concentration of IgM in calves supplemented with this vitamin (2000 / 27000 IE/day) was however significantly higher [493, 494]. Reddy *et al.* also concludes that vitamin E (125 IE/day) improves the immune response in calves and is therefore economically viable for this indication [495]. Carter *et al*. used the costs associated with antimicrobial drug therapy as indicator for effect of vitamin E on the immune response. They concluded from a study in 694 calves that vitamin E in a concentration of 2000 IU given during 28 days results in a decrease of medicinal costs of 22.4%. The overall economic outcome was positive ($0.38/hd) when the higher vitamin E concentration was used [496].

Supplementing pregnant cows with vitamin E will increase the plasma concentration of vitamin E in calves borne, but has no influence on the immune system of these calves [486]. The parenteral (subcutaneous) administration of 3000 IU vitamin E ten and five days prior to expected calving resulted in an increased efficacy of neutrophils to kill bacteria at the moment of calving. The oral administration of 1040 IU per day during the dry did not have any effect on neutrophil activity [497].

The use of vitamin E to stimulate growth of calves is controversial. In the chapter of Casals & Calsamiglia it is mentioned that vitamin E stimulates the production of antibodies and enhances the immune response and that an optimum intake of this vitamin brings about a lower incidence of retained placentas and metritis, improvements in the mammary gland health and the health of the reproductive system [311]. In one study the long term supplementation of vitamin E resulted in an increased growth rate [498], but the growth of calves could be stimulated with the use of vitamin E in two other studies [489, 499]. Pehrson *et al.* concludes that a deficiency of vitamin E will impair growth, but that supplementing will not increase growth further when the feed already contains enough vitamin E to meet the requirements [500]. The same conclusion is drawn by Casals & Calsamiglia after analysis of several articles [311]. A study by Bass *et al*. showed that supplementation of vitamin E during late gestation did result in higher plasma concentrations of vitamin E in the progeny compared to non-supplemented cows. Bodyweight at weaning was higher for calves from supplemented cows during winter. This effect was not seen during summer [501]. Cano *et al* mention that the effect of vitamin E on growth is indeed not consistent, which may be explained by differences in previous nutritional status, including vitamin E status, dietary vitamin E intake and stress [502]. For example, vitamin E could be used to enhance growth in calves which were exposed to IBR, but not in calves which were not exposed to this virus [489, 502].

The vitamin E status has also been linked to the occurrence of left abomasum displacement in early lactating cows. The cows developing left displaced abomasum (LDA) had plasma α-tocopherol concentrations 45% lower than healthy animals before the diagnosis of LDA. These concentrations remained low up to 49 days after parturition [503].

Vitamin E also influences meat quality. Supplementing calves at the end of the fattening period results in higher concentrations of vitamin E in the meat and as a consequence a reduction of lipid oxidation, a better colour and a better shelf life [311].

3.1.16 Vitamin K / menadione

Both in the literature and the NRC no information can be found about the recommendations for vitamin K in cattle or the beneficial effects of supplementing this vitamin. In veal calves an amount of 2 mg vitamin K / kg of DM milk replacer is advised by the INRA[443].

Vitamin K is prevalent in vegetables and ruminal bacteria and therefore deficiencies in ruminants are almost never detected. They are only reported in situations where anticoagulant substances have been consumed [311].

3.2 Minerals & Trace elements

3.2.1 Calcium

The requirements in dairy cattle depend on the phase in production:

- Fresh cows: 0.65-0.80%
- Lactating cows (90 days in lactation): 0.63-0.67%
- Close-up diet: 0.45%
- Close-up diet, anionic: 0.6-1.5%
- Close-up diet heifer: 0.40-0.44%
- Non-producing pregnant cows 240, 270 and 279 days pregnant: 0.44%, 0.45%, 0.48%

In young calves the following recommendations are made: 1.00% in milk replacer, 0.70% in starter feed, 0.60% in grower feed and 0.95% in whole milk[347].

For beef cattle the requirement of calcium also depends on the phase in production: 0.17-0.19% in the middle 1/3 of pregnancy, 0.25-0.27% during the last 1/3 of pregnancy and 0.24-0.31% during the first 90 days after calving. For pregnant heifers the recommendations are 0.35-0.53%, 0.26-0.39% and 0.26-0.42% for respectively the second and third 1/3 of pregnancy and the first 90 days of lactation. For growing calves, yearlings and mature bulls the recommendations are respectively 0.23-1.18%, 0.19-0.72% and 0.16-0.36% [411].

In young animals the calcium demand is higher than in full grown animals because of the storage of calcium in the skeleton. The same goes for pregnant cows 190 days or more in gestation, because the skeleton of the foetus than starts to calcify, and for lactating animals which need calcium for the production of colostrum and milk. During early lactation nearly all cows are in negative calcium balance, but when the feed and calcium intake increases cows often go into a positive calcium balance after 6-8 weeks. During the first 10 days most cows have a subclinical milk fever, but in severe cases clinical milk fever with a loss in nerve and muscle function can develop.

Calcium is needed for the formation of skeletal tissues, transmission of nervous tissue impulses, excitation of skeletal and cardiac muscle contraction, blood clotting and for the production of milk. Intracellular calcium is also important for enzymatic function and intracellular communication. When

animals are deficient of calcium, calcium will be withdrawn from the bones, but in time this will result in osteoporosis and sometimes even spontaneous fractures [347, 411].

In an old study it was shown that calves suffering from diarrhea have a lower plasma concentration of calcium, phosphor and albumin than healthy calves [504].

3.2.2 Phosphorus

The requirements in dairy cattle depend on the phase in production:

- Fresh cows: 0.34-0.42%
- Lactating cows (90 days in lactation): 0.32-0.44%
- Close-up diet: 0.3-0.4%
- Close-up diet, anionic: 0.3-0.4%
- Close-up diet heifer: 0.3-0.4%
- Non-producing pregnant cows 240, 270 and 279 days pregnant: 0.22%, 0.23%, 0.26%

In young calves the following recommendations are made: 0.70% in milk replacer, 0.45% in starter feed, 0.40% in grower feed and 0.76% in whole milk[347].

For beef cattle the requirement of phosphorus also depends on the phase in production: 0.14-0.15% in the middle 1/3 of pregnancy, 0.16-0.17% during the last 1/3 of pregnancy and 0.17-0.21% during the first 90 days after calving. For pregnant heifers the recommendations are 0.16-0.22%, 0.17-0.22% and 0.18-0.26% for the second and third 1/3 of pregnancy and the first 90 days of lactation respectively. For growing calves, yearlings and mature bulls the recommendations are respectively 0.14-0.52%, 0.12-0.34% and 0.11-0.20% [411].

Phosphorus is a very important mineral for animals. About 80% is incorporated in bones and teeth together with calcium. Phosphorus is important for almost all energy transactions that involve formation or breaking of high-energy bonds that link oxides of phosphate to carbon (nitrogen) such as ATP. Another important role is participating in the acid-base buffer systems of the blood and other body fluids.

Signs of a phosphorus deficiency include unthriftiness, inappetance, poor growth and lactation performance and unsatisfactory fertility. Severe deficiencies can result in bone mineral mass loss and rickets in young animals and osteomalacia in adults.

The Agricultural and Food Research Council has developed an equation from data found in the literature to calculate the absorbed phosphorus requirement for growing animals: P (g/day): $(1.2 + (4.635 \times MW^{0.22}) (BW^{-0.22}) \times WG$, where MW is the expected mature bodyweight and WG the weight gain.

The requirement of phosphorus during the final stage of pregnancy (>190 days) increases due to calcification of the foetal bones. Before 190 days the phosphorus requirement of the foetus is very low.

In the NRC guideline for dairy cattle several studies are discussed in which varying dietary concentrations of phosphorus were investigated in calves. A phosphorus percentage of 0.20% was not enough for growth of dairy heifers aged 3-18 months. Calves weighing 90-125 kg grew best with dietary phosphorus concentrations of 0.24%, but bone ash content was greater with 0.33%. An increase in phosphorus concentration from 0.22 to 0.32 did result in an increased serum concentration, but had no effect on weight gain or feed efficacy. In another study an increase of 0.24 to 0.35% or 0.26 to 0.34% did however result in an increase in dry matter intake, average daily gain, breaking strength of

ribs and tibia and blood plasma concentrations. Comparing 0.24, 0.30 and 0.36% resulted in the conclusion that 0.30% was optimal for feed intake and growth. Based on these studies, a concentration of 0.30-0.34% is advised.

Also studies concerning lactating cattle are reviewed in this guideline. Increasing phosphorus concentrations (range: 0.24-0.65%) did not result in a significant increase in dry matter intake or milk production in the first weeks of lactation. There might however be an increase in milk protein percentage with increasing phosphorus concentrations. Supplementing phosphorus at a concentration of 0.42% during the first eight weeks of lactation did however maximize longer term milk production.[347]

3.2.3 Sodium

The requirements in dairy cattle depend on the phase in production:

- Fresh cows: 0.26-0.34%
- Lactating cows (90 days in lactation): 0.19-0.22%
- Close-up diet: 0.1%
- Close-up diet, anionic: 0.1%
- Close-up diet heifer: 0.12-0.13%
- Non-producing pregnant cows 240, 270 and 279 days pregnant: 0.10%, 0.10%, 0.14%

In young calves the following recommendations are made: 0.40% in milk replacer, 0.15% in starter feed, 0.14% in grower feed and 0.38% in whole milk[347].

For beef cattle the requirement of calcium in growing and finishing animals is 0.06-0.08%. In cows the requirement during gestation is also 0.06-0.08%, but during early lactation the requirement is increased to 0.10% [411].

Sodium, together with chlorine and potassium, is important for important physiological functions such as the modulation of extracellular fluid volume and the acid-base equilibrium. Also heart function and nerve impulse conduction and transmission depend on the balance of sodium and potassium. Sodium is also important for Na-K ATPase, responsible for the creation of electric gradients for transport of glucose, amino acids and phosphate into the cells and hydrogen, calcium, bicarbonate, potassium and chloride out of the cells.

A deficiency of sodium might result in loss of appetite, rapid loss of body weight, lustreless eyes, rough hair coat, incoordination, shivering, weakness, dehydration and cardiac arrhythmias [347, 411].

4 Sheep

4.1 Vitamins

4.1.1 Biotin

The NRC does not mention minimal requirements for this animal species because biotin can also be produced in the rumen. It is however not clear if the ruminal production can meet the requirements.

No scientific studies can be found that have studied the biotin requirements of sheep and lambs or that have studied the effect of supplementing biotin in these animal species.

4.1.2 Choline

The requirement according to the NRC is 21 mg/kg bodyweight, but more research would be needed to confirm this requirement. It is also mentioned that a minimal amount of 2 gram per day would be needed to increase the daily weight gain in lambs [505].

Supplementing lambs with choline in quantities of 0.25-1% results in an increase in daily weight gain, but without alteration of the feed efficacy [380].

Choline also influences the rumen; supplementing choline (0.5, 1, 2 gram/animal/day) results in a decrease in the concentration of volatile fatty acids in the rumen and a slight increase in the digestion of dry matter [506].

4.1.3 Folic acid

Little research has been done because folic acid is synthesized in the rumen. They however do have some minimal requirements: 3.3 mg/ 100 kg bodyweight and 0.3-2.5 µg/ kg dry matter [505].

The clinical signs of a deficiency of folic acid in lambs have been studied in an old study. These are diarrhea and leukopenia. According to the authors the fact that signs of a deficiency can be induced means that folic acid is an essential part of the feed of lambs [507].

In a more recent study the effect of supplementing 5 mg/day in ewes in late pregnancy has been determined. There was no change in haemoglobin, haematocrit, glucose, lactate, sodium or potassium in the blood plasma or cardiovascular functions of ewes or their lambs [508].

4.1.4 Niacinamide

Niacinamide is not mentioned by the NRC, but niacin is. Niacinamide is an amide from niacin. This vitamin is produced microbiologically in the rumen of sheep. There are however studies in which it is shown that supplementing niacin to sheep will result in better production parameters and can play a role in the prevention of ketosis. A positive effect on the protein synthesis in the rumen has also been found in some studies, but not in all. The conclusion drawn is that the utility of niacin is doubtful [505].

Supplementing nicotinic acid in the feed of sheep results in a higher microbial production of niacin in the rumen. Ingested niacinamide will be hydrolysed tot nicotine acid and ammonia in the rumen [509].

When sheep are supplemented with niacin for a period of at least four weeks there will be a transition of type I to type II muscle fibres. These type II fibres are better capable of utilizing fatty acids. This is particularly meaningful during periods of metabolic stress; when large amounts of fatty acids are mobilized form the adipose tissues [407]. Another study also showed that niacin can be of importance in ketogenic situations. The mechanism discussed here however functions at the level of the rumen:

adding niacin (0,5 or 1 gram /day) results in an increase in the concentration of propionic acid and a decrease in the concentration of butyric acid [408].

4.1.5 Vitamin A / retinol

The NRC refers to several studies in which the requirement of vitamin A in sheep has been determined but a requirement of 104.7 IU/kg of bodyweight seems most reliable. Clinical signs of a vitamin A deficiency in sheep are: ulceration of the cornea, blindness, growth retardation, a loss of weight, weakness, incoordination and paralysis of the hind legs [505].

Growth of lambs in improved when the concentration of vitamin A in the feed is increased, both with feed with a normal or a low energetic value [510]. In a more recent study authors however found that supplementing 500.000 IU per animal twice a week (0-100 days of age) had no influence on the growth and meat composition, but there was an increase in the amount of adipocytes in peri-renal fat tissue which indicates hyperplasia and hypertrophy of adipose tissue [511].

Supplementing extra vitamin A to the diet of sheep improves the immune reaction against *Melaphagus Ovinus*; sheep that are given feed with a high concentration of vitamin A were less often infected with these flies and their larvae than sheep fed concentrate with a lower vitamin A concentration [512].

Recently a study has been done to determine the effect of vitamin A supplementation in pregnant ewes. It is however difficult to determine the effect of a temporary supplementation because sheep ingest large amounts of vitamin A when grazing and these vitamins can be mobilised form the liver during pregnancy [513].

4.1.6 Vitamin B_1 / thiamine

Thiamine is not well studied because it is produced microbiologically in the rumen. It is however also known that a deficiency can be the cause of polio-encephalomalacia (cerebral necrosis). Supplementing 1000 gram of thiamine would be sufficient to prevent the subclinical form of this disease, but there is no generally accepted minimal required amount of thiamine for sheep [505].

There is only one study available where the authors have investigated the effect of supplementing thiamine in sheep. They concluded that providing a higher concentration of thiamine will result in a higher production of propionic acid in the rumen [514].

4.1.7 Vitamin B_2 / riboflavin

In sheep with an active rumen vitamin B_2 is produced by the microbes. In goats it is shown that supplementing extra vitamin B_2 does not result in an increased excretion or increased ruminal production of this vitamin, which indicates that supplementation is not needed in adult animals. Milk replacers do need to contain vitamin B_2, but there is no minimum requirement [505].

A vitamin B_2 deficiency can be induced in lambs when a diet without this vitamin is fed. This results in a decreased feed intake, growth retardation and an increased occurrence of pneumonia. Supplementing vitamin B_2 (injection or 3 mg/kg in the feed) resulted in a fast improvement of these lambs [515].

4.1.8 Vitamin B_5 / D-panthenol

D-panthenol is not mentioned specifically by the NRC. Pantothenic acid is mentioned, but no minimal requirement is known for sheep. Pantothenic acid is produced in the rumen, but possible not to the extent in which it is needed. When the requirement of piglets is extrapolated to the requirements of lambs (of 45-50 kg), an amount of 14.84 mg per day would be needed. Microbial synthesis in the rumen probably does not meet this amount [505].

4.1.9 Vitamin B_6 / pyridoxine

It is expected by the NRC that the requirement can be extrapolated from these known for piglets. If this is true, the microbial synthesis in the rumen is twice as high as the required amount. There should however be more research conducted on the beneficial effects of supplementing higher concentrations of this vitamin [505].

4.1.10 Vitamin B_{12} / cyanocobalamin

Vitamin B_{12} is synthesised in the rumen and the amount produced would be enough, provided that there is enough cobalt in the feed. Cobalt is the main component of vitamin B_{12}. If this cannot be guaranteed, supplementation could be beneficial [505]. One old study showed that in animals with a properly functioning rumen 11 µg/day would be required. In young lambs this is not needed if the ewes have enough vitamin B_{12} in their milk. In sheep given a diet deficient of cobalt, the vitamin B_{12} requirement was 11 µg/day [516].

A deficiency of vitamin B_{12} can result in abnormalities of the liver: pale and brittle liver with infiltration of fat [505, 517]. It can also result in neurological signs and muscular disorders.

The concentration of vitamin B_{12} in the blood drops when lambs are fed a diet with low concentration of cobalt [518], which suggests it is an essential vitamin.

Supplementing vitamin B_{12} to ewes during pregnancy results in an increased concentration of this vitamin in the livers of lambs and in the milk of the ewes. The amount of vitamin B_{12} required by the lambs in the first thirty days of live can be provided in this way [519].

When vitamin B_{12} (0.06 mg/kg DM) is given to ewes before pregnancy the amount of corpora lutea increases and the lambs that are born from ewes which are supplemented are more active during the first three days of live [520]. A positive effect on the fertility of ewes is also shown by Fisher: ewes that were not supplemented with cobalt had less lambs and delivered more stillbirths. Also the mortality of lambs during parturition increased and the lambs were less vital which resulted in the fact that they waited longer to start suckling [521].

Vitamin B_{12} is also of importance for the immune system; lambs supplemented with vitamin B12 had a lower egg count after a natural infection with gastrointestinal nematodes than lambs that were not supplemented with this vitamin [522]. It is suggested that 21-100 mg of cobalt should be supplemented to each anthelmintic treatment in sheep [523].

4.1.11 Vitamin C / ascorbic acid

There is no NRC recommendation for vitamin C, because it is generally assumed that ruminants produce enough vitamin C to meet their requirements. In calves it is however known that they do not produce vitamin C in the first three weeks of life and more research is needed to confirm or exclude the occurrence of this phenomenon in lambs.

Vitamin C can protect the pleura in animals that are affected with pneumonia; in contrast to inflammatory mediators, vitamin C will result in a decreased permeability of the pleura. As a result, the inflammation will be more localized. Additionally, vitamin C protects membranes against oxidative damage [524].

4.1.12 Vitamin D_3 / cholecalciferol

The vitamin D_3 requirement of ewes in early pregnancy is 5.6 IU per kg bodyweight. During late pregnancy an additional 213 IU per day is needed. During lactation an extra 760 IU/day is needed. Neonates do not require additional vitamin D_3, provided that the ewes had a sufficient intake of this

vitamin during pregnancy and lactation. Young sheep and goat need 54 IU per day for every 50 grams of growth per day. Animals that walk on pasture seldom have a vitamin D_3 deficiency, but lambs that are weaned early have an additional requirement of 20%. A deficiency may result in problems with the formation of bones. Severe deficiencies during pregnancy in the ewes can result in malformations in the lambs [505].

Kohler *et al*. recently showed that small ruminants are capable of producing vitamin D_3 in their skin, but that sheep are more dependent on oral uptake of vitamin D_3 than goats [525]. In a less recent study it was shown that both the oral supplementations of vitamin D_3 as well as the exposure to ultraviolet light are effective in sheep [526].

The administration of very high concentrations of vitamin D_3 (10-80 times the recommended dosage according to NRC) barely results in an increase in the plasma concentration and does not result in clinical changes [527].

During winter the concentration of vitamin D_3 in plasma of ewes can be lower than during the rest of the year due to the lack of exposure to sunlight. As a result the concentration of vitamin D_3 in tissues of lambs born from these ewes will also be lower. Supplementing 300.000 IE two months before lambing is enough for both the ewes and the lambs [528].

4.1.13 Vitamin E / alfa-tocopherol

The requirement of vitamin E in growing and pregnant sheep is respectively 10 and 15 mg/kg DM. The requirement however depends on the concentration of selenium in the feed; when the feed is deficient of selenium the requirement of vitamin E increases to 15-30 mg/kg DM. Recent research shows that these quantities would not be enough and that an amount of 5.3 mg/kg bodyweight would be needed [505].

The effect of vitamin E on growth of lambs varies between different studies; in some breeds there is a significant increase in growth while other breeds show a decrease in growth when the lambs are supplemented with 15 or 30 mg per kg of feed [529]. More recent studies showed there was no effect of vitamin E on growth [530, 531].

Supplementing vitamin E is also supposed to have an effect on the meat quality. Supplementing 200 IU of vitamin E per sheep per day resulted in an increased concentration of vitamin E in the meat, a lower concentration of saturated fatty acids and a higher concentration of monounsaturated fatty acids [532]). MacGlaflin *et al*. however proved that injecting sheep every two weeks with 15 or 30 IU per animal was insufficient to increase the concentration of vitamin E in the meat or liver of the animals [533]. The shelf life of the meat can be improved by supplementing lambs with vitamin E; there is a positive effect on the colour, the formation of methaemoglobin and the fatty acid oxidation [534]. Also Lauzurica *et al*. showed a positive effect of vitamin E on the shelf life of the meat but higher concentration were needed (1000 mg/kg feed) [535]. This concentration is also used by Guidera *et al*. These authors showed that feeding ewes this amount of vitamin E results in an increased concentration of this vitamin in the meat and a decrease in the oxidation of the meat [536]. Wulf *et al*. showed that supplementing each lamb with 1000 IU of vitamin E results in a better quality of the meat than supplementing 500 IU [537]. Research from other authors however shows that 287 mg / kg feed would be the optimum amount to protect the meat from oxidation [538].

The effect of vitamin E on the immune system is controversial. Vitamin E (15 or 30 IU every two weeks) did not result in a better immunity against parasitic infections [533] and the production of antibodies against *Chlamydia psittaci ovis* did not increase significantly after supplanting 1 gram of this

vitamin per week [539]. Another author however did find that vitamin E is important for both the humoral and cell mediated immune response. The required amount of vitamin E is however difficult to determine and dependent on the concentration of other vitamins in the feed [540]. A very recent study showed that the supplementation of lambs with 10 IU/kg bw resulted in a lower worm burden after an infection with *Haemonchus contortus* and a greater recruitment of innate effector cells to the site of infection. These results could not be obtained when the amount advised by the NRC (5.3 mg/kg bodyweight) was administered [541]. Another recent study showed that vitamin E supplementation results in a reduction in adult fluke burden and a lower lipid oxidation in the liver when lambs are experimentally infected with Fasciola hepatica [542].

The role of vitamin E on the immune system is possible explained by the protection of biological membranes against oxidative damage. This is particularly important when the immune system is active and large amounts of free radicals are released. A vitamin E and/or selenium deficiency results in a decreased immune response and growth impairment [543]. For supporting the immune system 10IE of vitamin E/kg bw would be needed [505].

Supplementing vitamin E to rams (100-200 IU/day) results in a higher concentration of vitamin E in the testis and an increased enzymatic activity [544]. Yue *et al*. showed that 200 IU of vitamin E per day results in an improvement of the sperm quality and the amount of sperm produced. This is caused by the protection of testicular membranes and mitochondria against anti-oxidants. The optimum concentration of vitamin E in a supplement however has to be determined [545].

Giving vitamin E to ewes during late pregnancy (40 IU /day) does not change the birth weight of the lambs or the production of colostrum. Lambs from ewes that were given the supplement were less capable of absorbing antibodies from the intestines, which results in a lower plasma concentration of these antibodies [546]. Another study however does show that the bodyweight of lambs is increased when ewes were given extra vitamin E (500 mg/kg feed) [547].

When ewes lamb early during the season, supplementing 330 IU vitamin E per ewe per day leads to a lower mortality of the lambs and therefore to more kilograms of bodyweight produced per ewe at the moment lambs are weaned. There were no effects on the bodyweight or condition score of the ewes. When ewes that lambed later during the season were given the same amount of vitamin E there was no effect on the mortality of lambs [548]. Also Rooke *et al*. mention that supplementing vitamin E in pregnant ewes is beneficial, but more research is needed to determine the amount needed [513]. More recently this author showed that supplementing ewes with 250 IU of vitamin E does not have an effect on the body weight and vitality of the lambs [549].

A vitamin E deficiency in sheep results in a myopathy and hepatic lipidosis [550]. When lambs receive too little vitamin E and too little selenium, white muscle disease may occur. These lambs react best to a supplementation with vitamin E [551]. For the prevention of this disease a combination of vitamin E and selenium is best [552]. A deficiency of vitamin E also results in a decreased stability of erythrocytes. Supplementation of 300 mg of vitamin E will result in a quick clinical improvement in this case [553].

4.1.14 Vitamin K / menadione

It is expected that enough vitamin K is produced in the rumen and that supplementation of vitamin K would only be needed in ruminants that have ingested vitamin K antagonists [505]. There is no literature available about the requirement of vitamin K in sheep or the effect of supplementation.

5 Goats

5.1 Vitamins

5.1.1 Vitamin A / retinol

The supplementation of vitamin A to goats challenged with 3-methylindole to induce respiratory disease does not result in less clinical signs, but it does result in significantly less severe microscopic lung laesions [554].

5.1.2 Vitamin B_1 / thiamine

The ability of thiamine to protect goats from lead intoxication has been evaluated. The lead concentrations in blood, liver, kidney and brain samples were significantly lower than in goats that did not receive thiamine, but were still higher than those of control animals. Additionally, there were no differences in clinical laesions [555].

5.1.3 Vitamin B_{12} / cyanocobalamin

Vitamin B_{12} injections with an interval of eight weeks results in an increased final live (+13,.4 kg) and carcass weight (+8.3%) of male goats given a diet low in cobalt [556].

Al-Zadjali *et al* showed that goats with lower vitamin B_{12} serum concentrations had higher coccidial counts than goats with higher serum concentrations. They however also found an age difference in serum vitamin B_{12} with the lower levels in younger animals [557].

5.1.4 Vitamin E / alpha-tocopherol

The role of vitamin E as an antioxidant has been extensively studied in other animal species. There is also some prove of this efficacy in goats. Das *et al* and Mohanta *et al* showed that vitamin E is helpful in reducing the burden of arsenic induced oxidative stress [558, 559].

Suppletion of vitamin E in combination with selenium in kids milk replacer results in higher serum total proteins, globulin, red blood cells, IgA, IgG and IgM [560].

Vitamin E, when given in combination with selenium, can also improve milk production and quality in milking goats [561]. In male goats vitamin E supplementation can stimulate the development of reproductive organs. In this study sodages of 0,80, 320 and 880 IU per kid per day were evaluated. The dosage of 880 IU showed no benefits above the dosage of 320 IU [562]. The same concentrations can possible protect the testis from oxidative damage [563]. Also in female reproduction vitamin E can be important. The use of intravaginal sponges for oestrus synchronization can cause oxidative stress. Vitamin E supplementation during the preovulatory period can prevent the overproduction of reactive oxygen species and may improve the multiple birth rates of kids [564].

Myodegeneration in kids is also associated with a defieciency in selenium and vitamin E. In the aetiology of a specific case either one can be deficient, but supplementation of both is advised. Kids seem more prone to this disease than calves and lambs [565].

6 Horses

6.1 Vitamins

6.1.1 Biotin

Biotin has been studied as a supportive treatment in over 40 horses with hoof defects. These authors concluded that the supplementation of biotin in a dosage of 10-30 mg per day for a period of six to nine months is a useful treatment to support other remedial measures in such cases. Varying degrees of improvement in the hardness, integrity and conformation of the hoof horn were observed in all cases [566]. The long term supplementation of 0.031-0.037 mg/kg bodyweight is also well tolerated in horses and improved the horn quality of the growing hoof as it had not been possible before with other measures [567].

6.1.2 Vitamin A

Both a vitamin A deficiency and intoxication will impair growth. A mild deficiency also results in impaired haematopoiesis and a decrease in serum concentrations of iron, albumin and cholesterol. The standard advice of 12 µg/kg for weanling horses seemed too little: 16.8 µg/k is appropriate for maximal growth, 64.8 µg/kg for liver secreted serum constituents and 120 µg/kg for red blood cell criteria [568].

6.1.3 Vitamin C

The vitamin C supplementation in investigated for its anti-oxidative effects in horses with lower airway disease. Animals were supplemented with 15 mg/kg selenium and 30 mg/kg ascorbic acid. This combination had beneficial effects on the clinical outcome and anti-oxidant balance in horses with acute and chronic lower airway disease [569].

6.1.4 Vitamin E

Vitamin E is considered important for the immune system. Because the immune system function declines with age in horses, Petersson *et al* investigated the effect of vitamin E supplementation (15 IU/kg bw) orally on the immune system. They found that the bacterial killing capacity of monocytes and neutrophils increased and horses had greater serum IgG. There was however no effect on IgM production and the neutralizing antibody response to vaccination against West Nile virus [570]. Baalsrud and Overnes found that vitamin E or vitamin E and selenium supplementation results in an increase in humoral immune response to antigens (e.g. tetanus toxoid, equine influenza virus), but not on antibody titres against *E.coli* [571].

A vitamin E deficiency, in and of itself, does not appear to reliably cause disease in horses. Studies examining the effect of vitamin E deficiencies in exercising or resting horses have revealed no apparent clinical signs resulting from vitamin E deficiency. There are however three specific diseases that consistently have been associated with vitamin E deficiency: Equine motor neuron disease (EMND), equine degenerative myelo-encephalopathy (EDM) / neuroaxonal dystrophy (NAD) [572].

Vitamin E also plays a role in equine motor neuron disease (EMND); together with a lack of access to pasture and excessive dietary copper, a vitamin E deficiency is a likely risk factor for EMND [573]. Also in another trial the role of vitamin E deficiency in EMND is described. The mean plasma vitamin E concentration in EMND cases was significantly lower than that of control horses with the likelihood of the disease increasing as the vitamin E concentration decreased [574]. Mohammed *et al* were able to induce EMND in horses given diets low in vitamin E [575].
Vitamin E-deficient myopathy characterized histologically by a moth-eaten appearance in the mitochondria and anguloid myofiber atrophy in frozen sections of sacrocaudalis dorsalis medialis muscle biopsy specimens was found in horses with clinical signs of EMND that were highly responsive

to vitamin E treatment. This myopathy may be a specific syndrome or possibly precede the development of neurogenic muscle fibre atrophy typical of EMND [576].

In a case study Mayhew *et al* discussed that on a farm with a high incidence of equine degenerative myeloencephalopathy (EDM), horses had low vitamin E status. Vitamin E supplementation resulted in a correction of the deficient state in most horses and was associated with a drastic reduction in the incidence of EDM from 40% to less than ten percent in one year [577]. Also in another study this relation was evident: low plasma concentrations of vitamin E are a factor in the development of EDM in the first year of life of hereditarily predisposed foals [578].

Dietary supplements with vitamin E and selenium increase the red blood cell resistance to peri-oxidative stress induced in vitro and the glutathione peroxidase activity in lymphocytes increased. The malondialdehyde (MDA) concentration in plasma of racehorses and the mobilisation of low molecular weight anti-oxidants following physical exercise in race horses decreased [579].

In a case study it was described that six Mongolian wild horses developed a degenerative myelopathy. All horses in the herd had a vitamin E deficiency, but the plasma concentration was even lower in the affected animals. This suggest a role in the pathogenesis [580].

Besides vitamin C, also the effect of vitamin E in race horses under intense training has been evaluated. In conclusion, supplementation of 1400 IU per day resulted in maintenance of the general oxidative status [581]. A combination of vitamin E and selenium seemed to have an effect on the calcium, potassium, copper and iron concentration in serum and the copper/zinc ratio in horses subjected to strenuous exercise[582]. Williams and Carlucci however concluded that vitamin E supplementation had no effect on the oxidative stress in intensely exercised horses when horses already received normal vitamin E concentrations via their feed [583]. Also Siciliano *et al* found that vitamin E supplementation did not influence the integrity of skeletal muscles in exercised horses [584].

6.2 Minerals

6.2.1 Copper

An excess of copper can be dangerous for the animal, which is proven by the case study from the Netherlands, where hepatic copper accumulation resulted in a haemolytic crisis and euthanization [585]. High copper concentrations are also suggested in the pathogenesis of equine motor neuron disease (EMND); the concentration of copper in the spinal cord is higher in horses with EMND than in healthy horses [586].

6.2.2 Phosphorus

The phosphorus requirements found in 1974 for a 200 kg horse growing 1 kg per day is 100-166 mg/kg/day [587].

The absorption of phosphorus from the gastro-intestinal tract of the horse is very efficient [588].

A study from 1989 showed that providing horses with a low (0.24-0.35%), normal (0.68%) or high (0.95-1.06%) concentration of phosphorus did not influence productivity, blood mineral concentration or the occurrence of musculoskeletal abnormalities in weanling horses [589].

6.2.3 Magnesium

The magnesium requirements found in 1974 for a 200 kg horse growing 1 kg per day is 12-18 mg/kg/day [587].

7 Rabbits

7.1 Vitamins

7.1.1 Biotin

A deficiency of biotin in rabbits can result in loss of hair and dermatitis. The NRC however does not have any references for the biotin uptake [590].

7.1.1 Choline

A deficiency in choline in rabbits can lead to retardation in growth, a fatty and cirrhotic liver and necrosis of kidney tubules. After a prolonged choline deficiency a progressive muscular dystrophy can occur. In one study it has been shown that adding 1.2 gram of choline per kg diet can prevent these clinical signs from occurring and therefore this is the nutrient requirement mentioned by the NCR for growing rabbits [590].

7.1.2 Vitamin A / retinol

The vitamin A requirement for growing rabbits is 580 IU/kg feed, while that in gestating rabbits is at least 1160 IU/kg feed. Vitamin A is usually obtained by rabbits as a pro-vitamin, namely carotene. The efficacy of rabbits to convert carotene in vitamin A is however unknown.

Signs of a vitamin A deficiency in rabbits include retarded growth, neural lesions, ataxia, spastic paralysis, xeropthalmia and impaired reproduction. Also a lack of appetite can occur [590]. Besides, vitamin A is important for the reproductive function of rabbits [591]. Impairment of reproductive function can be noticed before other symptoms of a deficiency can be found. A deficiency resulted in a decreased number of viable eggs per pregnant doe, resulting in a lower conception rate and smaller litter sizes. Rabbits borne had lower bodyweights [592]. Vitamin A is also plays a role in the stabilization of the acrosomal and plasma membranes in sperm after ejaculation [593].

Intoxication with vitamin A can however also result in reproductive disorders. Does fed a diet with 102,278 IU vitamin A per kg feed showed foetal resorptions, abortions, stillbirths, disclosed hydrocephalus, micro-encephaly and cleft palate [594].

Vitamin A is also important for the immune function. Pletsiyi and Askerov showed that the administration of vitamin A promoted an increase in the weight of lymphoid organs, thymus and spleen. It also stimulated the production of antibody forming cells by the cell and increased the serum antibody concentrations during immunization with antigens [595].

7.1.3 B-vitamins

The NRC mentions requirements of niacin and pyridoxine, which are respectively 180 and 30 mg/kg feed for growing rabbits [590].

Coprophagy seems to be an important method of ingesting niacin, riboflavin, pantothenic acid and vitamin B_{12} for rabbits. An old study showed that coprophagy provides the rabbit with respectively 83, 100, 165 and 42% more vitamins than would be available if the soft faeces were not consumed [596]. Also in the NRC guideline it is mentioned that coprophagy can partially or completely foresee in the vitamin B complex requirement [590].

Another factor influencing the metabolism of B vitamins is aflatoxins in the feed. The occurrence of these toxins in the feed result in an increase of the plasma concentrations of folate, but a decrease of the plasma concentrations of thiamine, vitamin B_6 and biotin by more than fifty percent.[597]

7.1.4 Niacinamide

It is however shown that niacin supplementation up to 11 mg/kg bodyweight results in significant improvement of growth. Also for thiamine supplementation can in some cases be required, since rabbits fed deficient diets for a prolonged period of time developed mild ataxia [590].

7.1.5 Vitamin B_6 / Pyridoxine

A vitamin B_6 deficiency in rabbits results in a decrease in growth rate, scaly skin of the ears, acrodynia of the nose, eyes and forepaws, convulsions, mild anaemia, prolong blood clotting time, sudden paralytic collapse, creatinuria and death in about 100 days. Death usually occurred during a collapse. The vitamin B_6 requirement of these rabbits was determined to be 39 mg per day [598].

Vitamin B_6 can be used to protect kidney cells from the nephrotoxic effects of gentamycin in rabbits. More research is however needed to confirm this and determine the dosage [599].

7.1.6 Vitamin C /ascorbic acid

According to the NRC rabbits do not require vitamin C in their diets [590]. This is supported by a very old study from 1956 in which it was shown that the vitamin C concentrations in the organs of rabbits were independent of the vitamin C concentration in the feed [600].

High levels of vitamin C (0.5 g/ liter drinking water) are however effective in inhibiting oxidative processes and improving the characteristics of fresh and stored rabbit sperm [601]. The combination of vitamin C and E resulted in improved viability and kinetics of spermatozoa with an increase in fertility rate which is almost significant [602].

Vitamin C can also be used in rabbits to attenuate or treat gentamycin induced nephrotoxicity. High oral concentrations of 250 mg/kg were used for a period 26 days [603].

Vitamin C is also known to reduce the production of free radicals and improve the semen quality of rabbits [604].

7.1.7 Vitamin D_3 / cholecalciferol

Although the vitamin D requirement for rabbits has not yet been determined, it is known that rabbits can develop rickets when give a vitamin D deficient diet. Vitamin D concentrations that are too high are however also not good; in two studies it was shown that 23,000 IU per kg feed can result in toxicity symptoms [590]. In rabbits with broken bones a single intravenous injection with vitamin D_3 had positive effects on fracture healing when mechanical strength of the bone was evaluated [605].

In a more recent study it is however shown that supplementing only vitamin D is not effective in increasing bone mineralization. This is only effective when combined with high calcium levels. Vitamin D given in rabbits suffering from inflammation-induced osteoporosis will however supress bone resorption and restore the metabolic balance [606].

Brommage *et al* showed that some rabbits fed a vitamin D deficient diet for a prolonged period of time could maintain normal phosphate levels while other rabbits developed and hypophosphatemia. In the latter rabbits this led to inadequate skeletal mineralization and classical signs of osteomalacia [607]. Bourdeau *et al* showed that rabbits with a chronic vitamin D deficiency can increase renal conservation of calcium and phosphorus, but still develop a mild hypocalcaemia and moderate hypophosphatemia [608].

7.1.8 Vitamin E / alfa-tocopherol

According to the NRC the vitamin E requirement of rabbits is 40 mg per kg bodyweight for growing, gestational and lactating animals. There is however no literature to support this requirement. There are only studies that prove 16.7 mg/kg feed is insufficient and that higher concentrations are needed [590].

A vitamin E deficiency can result in degeneration of the skeletal and cardiac muscles, paralysis and fatty liver [590]. Chou *et al* showed that a vitamin E deficient diets in rabbits results in the occurrence of severe muscular dystrophy and abnormal erythropoiesis associated with abnormal iron metabolism and sequestration of iron in the skeletal muscle [609].

Vitamin E can be used to protect cells from ischemia-reperfusion, in this study caused by clamping of the renal artery [610]. Also the toxic oxidative effects on erythrocytes that can be caused by iron injections to rabbits can be prevented by the use of vitamin E [611]. Also for vitamin A toxicosis it can be an effective treatment [612]. High vitamin E supplementation (240 mg/kg) can also improve growth performances, carcass weights and the oxidative stability of rabbit meat [613]. In another study supplementing 100 mg vitamin E per liter of water (adjacent to 60 mg/kg feed) also resulted in improvement of colour and lipid stability of rabbit meat [614]. In another study it was shown that vitamin E supplementation at the rate of 60 mg/kg feed as recommended by the NRC does not prevent the production ore reactive oxygen metabolites (ROMs) in plasma. In animals receiving 375 mg vitamin E per kg diet the production of ROM was lower and the lipostability of the muscles was improved [615].The increase in oxidative stability can be found both in raw and cooked meat. Castellini *et al* however did not find an improvement in weight gain or feed intake [616].

Steroid injections can cause osteonecrosis in rabbits. The supplementation of vitamin E (600 mg/kg feed) reduced the incidence of osteonecrosis [617].

Vitamin E also has membrane stabilizing functions and can influence haemolysis of rabbit erythrocytes caused by retinol [618].

Supplementing rabbits with four daily intramuscular injection of 100 mg vitamin E promotes the return of neutrophils to the circulation after chemotactic challenge. This may be done by reducing their adherence to endothelium [619]. Vitamin E supplementation to rabbits experimentally infected with enteropathogenic *Escherichia coli* results in an increased neutrophil and mononuclear inflammatory response to EPEC, but does not contribute to clearance of the infection. It also contributed to an acceleration of the epithelial cell proliferation in the ileal crypts [620].

High levels of vitamin E (200 mg / kg feed) are effective in inhibiting oxidative processes and improving the characteristics of fresh and stored rabbit sperm [601]. Also Castellini *et al* proved an improved oxidative stability of sperm when 200 mg vitamin E was given per kg diet [621]. Yousef *et al* found that vitamin E supplementation results in a reduction of free radicals and an improvement of rabbit semen quality [604]. The combination of vitamin C and E resulted in improved viability and kinetics of spermatozoa with an increase in fertility rate which is almost significant [602].

It has been shown that vitamin E concentrations in liver and muscles of rabbits with hepatic coccidiosis is lower than in non-infected animals, even when they do not have gross liver leasions [622].

7.1.9 Vitamin K / menadione

In the NRC book a nutrient requirement of 0.2 mg/kg feed is mentioned for gestation rabbits. Limited studies are available, but it is indicated that no vitamin K is needed in the diet for normal growth. Gestation rabbits receiving a deficient diet however developed placental haemorrhage and abortion [590].

8. Bees

8.1 Vitamins

As can be expected, little information is available about the vitamin requirement of bees. The requirements and the biological functions of vitamins in these insects are seldom investigated. With the literature search only two articles were found; one concerning vitamin A in vision and one about vitamin C in bees infected with *Varroa destructor*.

8.1.1 Vitamin A

It is demonstrated that bees have vitamin A in the tissues of their heads, but not in the thorax or abdomen. The concentration was higher in light-adapted bees; dark-adapted showed very low concentrations of vitamin A, but these concentrations increased when they are exposed to light. It is therefore assumed that vitamin A plays a role in vision [623].

8.1.2 Vitamin C

The effect of vitamin C supplementation (1.8 mg in syrup) on honeybees naturally parasitized with *Varroa destructor* has been studied very recently. These authors showed that supplementing vitamin C results in a lower prevalence and intensity of the infestation and strengthening of the anti-oxidative system [624].

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